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14. ABSTRACT
After meeting the Advanced Concept Technology Development (ACTD) original developmental milestone objectives, the portable molecular diagnostic (pMD) platform contract with Applied Biosystems (ABI) was terminated when the contractor was unwilling to seek FDA approval. ABI testing up to contract termination is included in this report. The EOS ACTD program initiated a technology review process and pre-validation testing to determine if other technology platforms could be developed in time to meet EOS ACTD objectives. This process has been summarized in a series of notebooks and is included in this report for each potential vendor and technology, including a composite Technology Recommendations report. Quarterly scientific meetings were held and the results and conclusions of the meeting presentations are discussed from the final Scientific Advisory Board (SAB) meeting held in Atlanta on July 31, 2007. Additionally, the Real Time Polymerase Chain Reaction (RT-PCR) testing performed at the Advanced Diagnostic Laboratory during the Lackland AFB Adenovirus type B14 outbreak including public health prevention and control testing strategies concludes the final report.

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TABLE OF CONTENTS

Contract Research Support of the
Epidemic Outbreak Surveillance (EOS) Program

INTRODUCTION

Epidemic Outbreak Surveillance Research; The contract research of epidemic outbreak surveillance (EOS) Program was conducted in support of the Air Force Modernization office (AF/SGR) at the Advanced Diagnostic Laboratory (ADL) at Lackland AFB, TX. The contract cost provided analytical laboratory equipment, supplies, Laboratory Information Management System (LIMS) equipment and infrastructure, laboratory construction/modification, equipment set-up, and manpower to operate the ADL. The ADL work-plan was established to provide an end-to-end system of real-time public health service in the basic military trainee beneficiary population for influenza like illness. A daily work-flow was established to allow for patient information capture through standardized sample collection, real-time sample analysis, data integration into laboratory information management database, and information reporting for pathogens in the human population.

SUMMARY

Lackland Advanced Diagnostic Laboratory Operating Capability; Under contracted research, levels 1, 3 and 5 medical treatment facilities were monitored to understand the technology requirements needed to meet the warfighters requirements at all levels of care. EOS contracted medical personnel collected clinical samples weekly from patients exhibiting upper respiratory symptoms including fever of 100.5^{o}F or higher. When a patient arrived with acute respiratory symptoms, a member of the clinical team was paged to collect specimens including a nasal wash, throat swab and blood from consenting patients according to standard protocols (Appendix A). Ten days to two weeks later, this set of samples was taken again from the same consenting, convalescent individual.

The functional utility of clinical collection was three fold. Clinical isolates exist in the native sample matrix that inherently includes all sample components present in that matrix including human proteins, carbohydrates and lipids, human nucleic acids and naturally-occurring inhibitors. Clinical samples also provided insight into the evasive question of clinically-relevant levels of pathogen concentration. Finally, isolates represented the most relevant pathogenic strains and sequences required to understand the detection needs of today. Current molecular and immunoassays must be able to recognize current pathogenic strains.

Clinical samples were delivered to the ADL and processed according to standard protocols (Appendix B). All nasal wash and throat swab washes were aliquoted and tested or stored at the ADL. Briefly, throat swabs were washed in 1ml of normal saline. Samples were vortexed and aliquoted into 6 microcentrifuge tubes of 100ul. Nasal washes were vortexed and aliquoted into 8 microcentrifuge tubes of 100ul. Blood was centrifuged and the serum stored until a matching acute and convalescent pair was obtained and shipped to the Centers for Disease Control and Prevention for further

testing. Through culture based testing, direct immunofluorescence and PCR, samples were screened for upper-respiratory pathogens of medical significance to this population. Pathogens in patient's samples were extracted using the Qiagen Biorobot, the MagNA Pure Compact or the 6100. Bacterial cell walls and viral capsids were lysed open and the nucleic acid bound, washed and purified of inhibitors then eluted. Real-Time Polymerase Chain Reaction (RT-PCR) wet and lyophilized assays were used to amplify and detect the nucleic acid. Detection and analysis was performed on the ABi7500 and 7900 utilizing molecular signatures provided by CDC to specifically amplify sequences within Influenza A, Influenza A-H1, Influenza A-H3, Influenza A-H5A (5.1 + 5.2) Influenza A-H5B, Influenza B, SARS.1, Respiratory syncycial virus, Adenovirus-pan (universal), Adenovirus subtype 4, Human Parainfluenza Virus 1, Human Parainfluenza Virus 2, Human Parainfluenza Virus 3, Human Metapneumovirus, *Streptococcus pneumonia*, *Streptococcus pyogenes*, *Mycoplasma pneumonia*, *Chlamydia pneumonia*, *Bordetella pertussis I*, *Bordetella pertussis II*, and *Legionella pneumonia*. Applied Biosystems Taq-Man Low Density Array (TLDA)/Silent Guardian Upper Respiratory (SGUR) cards 0.1, 1.0, 1.1, 2.0A and 2.0B (Appendix C) were used to screen samples from febrile, respiratory patients that tested negative by the AFIOH viral culture panel, followed by testing with individual CDC assays.

A 200ul aliquot of each sample was sent to the Air Force Institute for Operational Health for growth on tissue culture including primary monkey kidney (PMK) cells and A549 cells, and detection including visualization of cytopathic effect, hemagglutination testing and direct immunofluorescent antibody staining (Appendix D). The screening process allowed for the detection of adenovirus, enterovirus, herpes virus, influenza A, influenza B, parainfluenza viruses 1 and 3, respiratory syncycial virus. In addition, PMK and A549 cells were inoculated for 10 day growth to detect slow growing pathogens. A549 cells were used specifically for the growth of adenovirus.

Group A positive Streptococcal samples were obtained from the Wilford Hall Medical Center Microbiology Laboratory under an exempt protocol. The WHMC Microbiology laboratory identified isolates on the basis growth of Beta-hemolytic colonies on sheep blood agar. Isolates were then Lancefield typed by performing the Steptex procedure using a specific agglutination with latex particles coated with anti-group A streptococcal antibodies (Appendix E).

Through these efforts, the characterized, clinical samples were used for developmental testing of the most advanced molecular diagnostic and surveillance technologies, including the ABI pMD and ITI FilmArry platforms as described in this report. A spreadsheet of all EOS collected and characterized samples since January 1, 2007 are included in Appendix F.

IRB PROTOCOLS

All samples were collected under 5 unique IRB approved protocols.

1. Diagnosis, Surveillance, and Epidemiology of Upper Respiratory Tract Infections in Basic Trainees (Non-Exempt). Under this protocol, EOS clinical personnel collected specimens from consenting basic military Air Force trainees who were exhibiting febrile, respiratory symptoms. Samples were characterized for pathogens of concern in upper-respiratory tract infections.

2. Rapid Diagnosis of Respiratory Viruses in Hospital and Clinic Patients (Non-Exempt). Under this protocol, specimens were collected from patients presenting with febrile, respiratory symptoms at WHMC hospital or the Reid Clinic at LAFB and characterized for respiratory viruses and bacteria.

3. Obtaining specimens from control donors for establishing method validation and normal ranges (Non-Exempt). Under this protocol, nasal washes were obtained from healthy individuals who also consented for their samples to be used in research studies. The nasal washes were previously pooled, but this led to problems. The samples could not be vortexed, or mixed well-enough to get a homogenous distribution of all the commensal organisms in the combined matrix. To resolve this issue, all samples were labeled and individually characterized. Normal (negative) nasal wash and throat swab wash aliquots were spiked with American Type Culture Collection (ATCC) strains, purchased under this contract, and used to validate sensitivity and specificity of the Idaho Technology, Inc. FilmArray platform.

4. Identification of respiratory viruses by enzyme immunoassay (EIA), polymerase chain reaction (PCR) and Microarray Assays on Nasal Wash Specimens (Exempt).

5. Sequencing of clinical isolates of adenovirus associated with upper respiratory tract infection and development of clinically relevant probes/primers for molecular diagnostic tools (Exempt). Protocols four and five were written as exempt, which means that the samples were completely de-identified, so no link to the original patient exists. Samples were obtained from the WHMC microbiology laboratory that test positive for a pathogen of interest from rapid laminar flow assays.

6. CDC Human Adenovirus and Adenovirus Type 14 Specific Real-time PCR Protocol. During recent adenovirus events, this protocol was expedited by the WHMC Institutional Review Board (IRB) and used to test excess sample from diagnostic ordered test either at LAFB or from AFIOH on request for Adenovirus Type B14. This protocol was written in accordance with FDA Guidance on Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable - Guidance for Sponsors, Institutional Review Boards, Clinical Investigators and FDA Staff Document issued on April 25, 2006.

The Epidemic Outbreak Surveillance
Role and Capability for Adenovirus Type B14 Testing in
Public Health Prevention and Control at
Lackland Air Force Base, Texas

ADL Testing; During the adenovirus outbreak at LAFB, standard practice for EOS medical personnel was to perform a rapid adenovirus test on consenting patients exhibiting upper respiratory symptoms including fever of 100.5°F or higher. Adenovirus is a common cause of upper respiratory infection that usually manifests as cold symptoms, pharyngitis, or tonsillitis. However, many adenovirus infections at LAFB at this time progressed into pneumonia with lobar infiltrate, multilobar infiltrate and/or effusion, requiring hospitalization and in certain instances, intubation and mechanical ventilation.

Screening with the adenovirus rapid test allowed for a 15-minute designation of the presence of adenovirus in the patients sample with limited sensitivity. The adenovirus Sure-Vue rapid test is a membrane-based immunoassay that utilizes a monoclonal antibody against the group-reactive antigen of human adenovirus to qualitatively detect the presence of this antigen in adenovirus serotypes present in upper respiratory secretions.

EOS laboratory associates used Real-time polymerase chain reaction (RT-PCR) to confirm the presence of adenovirus and detect the presence of other pathogens within patient's samples. PCR is now the most commonly used method for user-developed assays, although only a few commercial kits incorporate this technology. At the ADL, samples were prepared for PCR by first extracting nucleic acids from the virion within the patient's sample. The Magna Pure Compact is a robot that performs manual extraction utilizing mechanical manipulation to lyse the nucleocapsids and magnetic beads to bind the DNA as it is washed, then eluted for further processing. The Center for Disease Control and Prevention provided the most current and relevant assays for the detection systems used at the ADL. The real-time PCR assay was performed using the Ambion AgPath-ID RT-PCR Kit with each 50 µl reaction mixture containing 25ul 2xRT buffer, 2ul 25x PCR enzyme, 15x Enhancer, and .25 µl of 25 µM forward primer, 0.25 µl of 12.5 µM reverse primer, 0.25 µl of 10 µM probe, and 20 µl of nucleic acid extract. Amplification was carried out in 96-well plates on an AB 7500 Real-Time Detection System. Thermocycling conditions consisted of 10 min at 95ºC for activation of the DNA polymerase, and 45 cycles of 15 s at 95ºC and 45 sec at 60ºC. Each run included one no-template control. CDC primer and probe sequences will be published in manuscript in preparation (Lewis P, Schmidt M, Gilbert D, Kendall B, Thomas A, Cieslak P, Erdman D, Lu X. Community-based cluster of severe Adenovirus 14 infection Lancet) as referenced and listed below.

Primer/Probe[a]	Sequence (5'- 3')	Amplicon length (bp)	Position[b]
Primer, fwd	GAA AAT CAT GGT GTG GAA GAT GAA	110	19443-19466
Primer, rev	CAA GCT TGG TCT CCA TTT AAC TGA		19552-19521
Probe	AC GGC ATC GGT CCG CGA ACA		19492-19591

[a]Probes 5’-end-labled with 6-carboxyfluorescein (FAM) and 3’-end-labled with Black Hole Quencher™
[b]Nucleotide numbering based on Ad14 (accession no.AY803294).

All adenovirus samples were subtyped for B14 on a daily basis to keep up with increased demand and to provide public health officers with the most recent adenovirus outbreak sample information. All rapid test results and PCR subtyping information was provided to WHMC officials and public health in real time to monitor and control the outbreak. A hot wash with the CDC epidemiology team and base commanders was held every morning and afternoon during the peak of the adenovirus incident.

To determine if co-infections are contributing to the pathogenicity of the adenovirus associated with the current LAFB outbreak, PCR was used to identify additional pathogens that may have been present in any of the IRB protocol-consenting patient’s samples containing Ad B14. In this instance, the samples were extracted and run on an EOS prototype card for RT-PCR. The primers and probes used to amplify and detect conserved sequences within each pathogen were lyophilized onto the card. Each card was used to run 8 samples at a time, and each sample was tested for 24 pathogens that also cause upper respiratory symptoms that in some instances mimic adenovirus infection. Pathogens tested included Influenza A H1, H3, H5A (5.1 and 5.2), H5B, Influenza B, Respiratory Syncycial virus, Human Parainfluenza 1, 2 and 3, Adenovirus (universal), Adenovirus E4, SARS.1, *Streptococcus pyogenes*, *Streptococcus pneumonia*, *Bordetella pertussis* (I) and *parapertussis* (II), *Chlamydia pneumonia*, *Mycoplasma pneumonia*, Human Metapneumovirus, and *Legionella pneumonia*. No evidence of associated co-infections with other pathogens was detected in samples from patients infected with adenovirus B14 at this time; however an alternate serotype of adenovirus, Ad4 may have been associated with B14 infection as reported by the Naval Health and Research Center.

CDC Epidemiology Studies; CDC and Texas Department of Health worked with Lackland Public Health officials and Base Commanders on several studies for the control and prevention of Adenovirus type B14 in Basic Military Trainees. Public Health testing for all associated studies at LAFB was performed by EOS personnel at the Advanced Diagnostic Laboratory. Out of four flights in the 6th week of training, 38% tested positive for B14; however when a female flight was taken into consideration the numbers dropped to 25%. Males historically have higher incidence of adenovirus than females. Only 5% of employees of Wilford Hall Medical Center at Lackland AFB tested positive for adenovirus type B14. The “fever flight”, now termed “bed-rest flight”, peaked at 60 BMTs and ranged from between 15 and 20 after EOS real time PCR testing was implemented early on in the outbreak. Wilford Hall Medical Center IRB approved a new EOS protocol for testing de-identified samples from AFIOH. This allows the testing of excess sample from a requested diagnostic test to identify new pockets of adenovirus that may be of significance to public health at outside facilities. This process is set up to be implemented during future pathogenic bio-threat events so that EOS testing of new prototypes will continue to be utilized to their full extent, allowing for better prevention and control measures to stay at the front end of an epidemic curve.

Adenovirus Environmental Surface/Swipe samples in BMT dorms; Samples were collected 2 July 2007 starting at 0500. The sampling team included Lt Brownheim, SSgt Price and SrA Ramirez of Bioenvironmental Engineering from the 37 AMDS/SGPB. The Sampling locations were as follows:

Area to be sampled	Male or Female Restroom
Squadron 331, flight 418, dorm A7 Bay	Female
Squadron 331, flight 484, dorm A2 Bay	Female
Squadron 331, flight 486, dorm A5 Bay	Male
Squadron 323, flight 508, dorm B10 Bay	Male
Squadron 322, flight 449, dorm B2 Bay	Male

Items sampled at each flight:

Item/Area to be sampled	Number of items to be sampled
Sink Spouts (outside/inside lip of spout)	2
Hot & Cold Sink handles	2 of each set
Urinal Handles & sides of urinal (men only)	2
Canteens (inside lip of spout)	2 random from each Bay area
Shower heads	2
Drain (inside drain lip area)	2
Door handle exiting the restroom	1
Toilet handles	2

Samples were taken immediately to the ADL following the completion of collection. Each item was thoroughly swiped, in the case of sink spouts, drains and shower heads, the inside of all of these items were thoroughly swiped using sterile swabs. After swiping, the handles of the swabs are broken off and the swab tips were put into a prepared vial containing appropriate buffer solution provided by the CDC.

Environmental samples were tested by RT-PCR as outlined in accordance with the ADL testing plan using the CDC Adenovirus Type B14 and Adenovirus Universal assays. All environmental samples tested negative under these protocols.

APPLIED BIOSYSTEMS TECHNOLOGY DEMONSTRATIONS

Introduction: The point of card medical device (pMD) concept, as funded by USAF as part of the Advanced Concept Technology Demonstration Spiral 1, was envisioned to deliver a solution to change the standard of care. The implementation of this goal was represented by a bench top device, including a sample preparation device and a signal detection device that is capable of delivering a result, from sample to answer, in less than 1 hour. A central feature of this concept, the pMD card, was designed to contain reagents in individual wells sufficient to enable multiple independent TaqMan assays at the same time. Included in this card design were wells for 20 pathogen specific assays, provided by CDC at the request of the USAF, and additional wells for both positive and negative controls. The benefit of this approach is that samples were run simultaneously against all assays, with a simplified work flow, and a general ease of use.

Experimental Outline: To determine if the TLDA and pMD cards were sensitive enough to detect pathogens on EOS ACTD Tier 1 list, they were tested against a panel of clinical samples from the ADL. Data on 24 matched nasal wash and swab samples from culture positive Flu A patients collected at the ADL was generated from this developmental study.

The results indicated that the FluA assay was positive for all 7 samples, and negative for the negative template control. The FluA-H3N2 assay was positive for 4/7 and weakly positive for the remainder. The signal from the internal control, RNAseP, varied between samples and may indicate variation in total nucleic acid extract from each nasal wash. The performance of H3N2 assay significantly lagged that of the FluA assay. Ideally, the cycle threshold (Ct) values should be identical (FluA = H3N2). This is especially important on the lower range of target concentration because subtyping is unreliable on the lower. A redesign of the H3N2 assay may correct this problem. Data is summarized in the table below.

	Influenza A		
	AB-TLDA	FLDOH-TLDA	pMD
F04274 (Nasal Wash)	27.19	26.87	23.35
F40366 (Nasal Wash)	24.12	24.52	23.36
F43541 (Nasal Wash)	25.38	25.27	22.31
F43632 (Nasal Wash)	29.64	29.13	26.08

In a second study, fifty six characterized samples from Lackland Air Force Base in the form of Nasal Wash and expressed Throat Swabs tested. 150ul of each sample was processed through the IDIS Purification Protocol at AB. Half of the resulting purified nucleic acid was shipped to Florida Department of Health Lab in Miami and the other half remained at AB. Results of the characterization were unknown to AB. However, based on previous conversations, presence of Streptococcus pyogenes, Human Adenovirus, and Influenza were expected. Nucleic Acid samples were analyzed on the SGURv1.0 TLDA cards at both sites in parallel. Simultaneously, 12 Nasal Wash/Throat Swab samples were also processed through the pMD Breadboard Sample Preparations System and analyzed on a pMD Card. There is good correlation between the pMD system

and the IDIS / TLDA system detection in all sets of experiments. The results of the studies are included in Appendix D and are summarized in the table below:

	Flu B			Adeno-pan			S. pyo			Flu A		
	TLDA-AB	TLDA-FLDOH	pMD	TLDA-AB	TLDA-FLDOH	pMD	TLDA-AB	TLDA-FLDOH	pMD	TLDA-AB	TLDA-FLDOH	pMD
FE6923	35.49	35.54	29.11									
FE4657	Controls Failed	23.51	23.73									
135870				37.54	37.49	29.34						
740886										26.41	25.65	24.26
PN001 (TS)												
214962							36.38	34.01	34.41			
361614				31.29	31.10	28.57						
214962 (TS)							30.37	30.47	31.72			
FE3497	31.41	29.59	26.53									
F43172												
626278				23.94	23.86	25.06						
F38123 (TS)							25.09	25.22	26.49			

Correlation between TLDA (AB) and TLDA (FLDOH) for Tier 1 pathogens:
53/56 (94.6%)

Correlation between TLDA (AB) and culture results for Tier 1 pathogens:
43/56 (76.8%)

Correlation between TLDA (FLDOH) and culture results for Tier 1 pathogens:
42/56 (75%)

Correlation between pMD (AB) and culture results for Tier 1 pathogens (12 samples):
10/12 (83.3%)

The testing and results of both AB studies are detailed in Appendix G.

IDAHO TECHNOLOGY, INC. TECHNOLOGY DEMONSTRATIONS

Technology Background – Film Array; Idaho Technology develops, manufactures, and sells machines for DNA analysis, including DNA amplification, real-time thermocycling, Hi-Resolution Melting, mutation scanning and genotyping. Their complements of products include the R.A.P.I.D. (Ruggedized Advanced Pathogen Identification Device) and the RAZOR instruments, the LightScanner, HR-1 and RapidCycler instruments, and the JBAIDS (Joint Biological Agent Identification and Diagnostic System). Freeze-dried reagents and DNA/RNA purification kits are available for each system. IT BioChem, a division of Idaho Technology, offers a complete list of buffers, probes, primers and melting dyes for their instruments and other real-time thermocyclers.

The Film Array is a technology that evolved from a Defense Threat Reduction Agency-funded project to aid in the development of the RAZOR instrument by Idaho Technology, Inc (ITI). The current prototype was developed under U-01 Grant from NIH NIAID. April 1, 2007 ends year 2 of a 4-year effort and a new award totaling $8.9 M was recently issued from NIH to include creation of a multi-sample Film Array instrument, Avian influenza identification and an expanded influenza typing assay panel. This initiative also includes a network-aware database and epidemiology system to provide immediate feedback to clinicians on pathogen prevalence.

Currently, instruments are produced by hand. An ITI 2007 Film Array goal is to transfer to instrument production to a cGMP compliant manufacturing production line. RTPCR sensitivity for RNA viruses on this platform has not been demonstrated, although lyophilized assay performance for bio-threat RNA viruses optimized at ITI (EEE, VEE, WEE, Ebola, Marburg) meets 10^4pfu/ml sensitivity.

Although the instrument weighs 20lbs, not including the laptop, ITI plans to redesign the instrument to reduce the amount of free volume which would not only reduce the size, weight and power reduction, but also serve to ruggedize the device in the process by adding additional insulation. Instruments available for Beta testing do not exist in this configuration.

The current capability of the Film Array prototype projects the instrument to detect greater than 50 viral or bacterial agents from a single sample, and to process clinical or environmental samples at JBAIDS sensitivity levels (10^3cfu/ml, 10^4pfu/ml). The instrument is expected to be commercially launched in December of 2007 with an upper respiratory panel. The panel includes assays for Adenovirus, HRV, PIV1, PIV2, PIV3, RSV, Influenza B, BOCAvirus, HMPV, Influenza A (Matrix, H3, N2, H5 and N1) and the Coronaviruses 229E, NL63, OC43, HKU1, and SARS. Sample is delivered into the fully enclosed system pneumatically into working blisters which contains sample preparation and PCR freeze dried reagents. Sample preparation and mixing is accomplished by electro-pneumatic integration via valves, air channels, pneumatic bladders and a high-pressure piston seal integrated into this single device, and all occurs within the small, enclosed pouch. The target time to sample identification is 45 minutes, however the current time to result as performed in the demonstration is 56 minutes: 17 minutes sample preparation, 34 minutes for amplification, 5 minutes for high-resolution melt analysis. The machine, laptop and a single patient reagent pouch weigh less than 25 lbs, and the instrument measures 8” x 11” x 17”.

First ITI Technology Demonstration at the ADL; A Film Array demonstration was performed at the Advanced Diagnostic Laboratory at Lackland Air Force Base by Cory Estes, Joanne Fisher and Kirk Ririe of ITI. A colony of *Saccharomyces cerevisiae* (Brewer's yeast) was picked and added to 100ul of reagent grade water. A sample volume of 100-200ul can be added by syringe into one side of the reagent pouch. Lysis buffer was then inputted similarly into the opposite side of the pouch. (The pouch and the lysis buffer are stable between 5°C and 40°C for greater than 6 months.)

Once in the reagent pouch, sample was delivered into a lysis pouch, or blister containing micro beads. The beads were agitated for 60 seconds, lysing the sample. Sample preparation required 18 minutes and occurred in adjacent blisters via magnetic particles. Following two wash steps, the nucleic acid was eluted into the next blister within the pouch and underwent RTPCR for 5 minutes at 50^{o}C followed by nested multiplex Polymerase Chain Reaction (nmPCR) with outer primers targeting 200bp segments. The primers were then split between multiple singleplex reactions. This is PCR stage 1 (PCR1). Cycling parameters of PCR1 included 20 cycles of zero seconds at 95^{o}C and 8 seconds at 63^{o}C. The purpose of PCR1 was to permit assays with demonstrated high sensitivity to run simultaneously in combination with a large panel of biological assays. A 100 fold dilution of the product occurred within the pouch following PCR1 to dilute out extraneous amplified product. PCR2 occurred in the wells of an array in the next blister utilizing inner primers specific for up to 120 assays (in current configuration) for amplification of approximate 90bp targets. The cycling parameters for PCR2 were 30 cycles at 95^{o}C for zero seconds, followed by 63^{o}C for 8 seconds. The probes used in the demonstration were double-stranded, florescent DNA dyes, allowing for an automatic melting point analysis at the end of the run. The cycling parameters of the melting point analysis were 70^{o}C and 95^{o}C with continuous acquisition for 4 minutes. The demonstration, including adding sample and conducting melting point analysis, took approximately 56 minutes. *S. cerevisiae* amplified as expected during the demonstration.

A complete set of controls was developed and being integrated into the reagent pouch including controls for fluorescein, empty cell, melt calibrators, PCR2 amplification, PCR1, RNA control and organism DNA/RNA. A control table is represented below.

	DNA in PCR 2	DNA in PCR 1	RNA assay in e.g. yeast	DNA at start but primers in PCR 2	Negative control
Bead beating			X	X	
Extraction			X	X	
RT			X		
PCR 1 amp		X	X		
Dilution		X	X	X	
PCR 2 amp	X	X	X	X	
Contamination					X

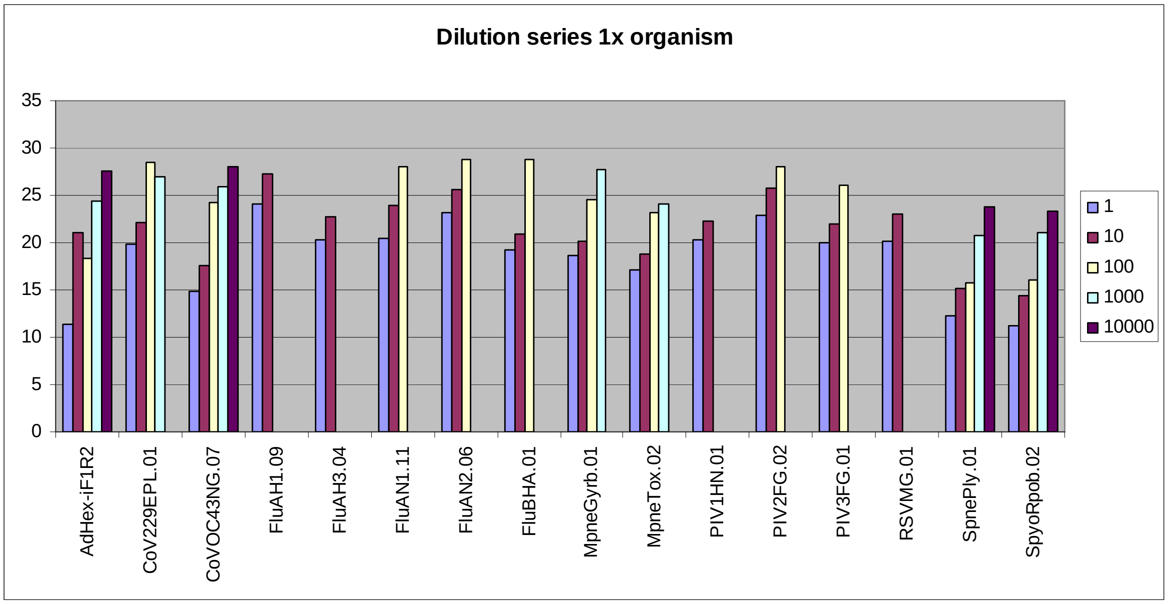
Lyophilized *S. cerevisiae* genomic DNA within the reagent pouch acted as a DNA control its spliced transcript acts as the RNA control. Primers for the DNA control were

only added to PCR2, controlling for bead beating, extraction, dilution and PCR2. The RNA transcript controlled bead beating, extraction, RT, PCR1, Dilution, and PCR2. DNA was present in PCR1 blister and controls for PCR1 amplification, dilution and PCR2 amplification. DNA is present in the blister with the array in PCR2 and controlled for PCR2 amplification. An empty well served as the negative control. Results and amplification curves of cycle threshold values are depicted in Appendix H.

Second ITI Technology Demonstration at the ADL; Detection of Respiratory Pathogens in Clinical Samples: 15ul from approximately 2ml nasal wash or 1ml throat swab washes were extracted in an integrated sample extraction-detection pouch on the ITI FilmArray. Clinical samples extracted, amplified and detected at this volume include RSV, S. pyogenes, Flu A (H3N2), and Adenovirus E4a. In a separate mix of 35ul each, PIV1 and H3N2 were detected but PIV3 was not. At a 100 and 150ul volumes, Adenovirus B14 and Flu B were detected, respectively. Negative samples did not amplify with the exception of a S. dysgalactiae sample that may be amplifying with S. pyogenes primers. S. pneumoniae was detected as a commensal.

Pin Number	Collection Date	Agent	Volume of Test 1 Mix	Test 2 Mix	Test 3 Mix (neg)	Test 4 Mix	Test 5 Mix	Test 6 Mix
FE8935 NW	7/25/2005	PIV 1	15ul					35ul
FE1353 NW	12/19/2005	PIV 3	15ul					35ul
FE1795 NW	1/14/2006	RSV	15ul					
F86289 NW	11/3/2005	S. pyogenes	15ul					
F98395 NW	12/19/2005	Influenza A	15ul					
F40366 NW	12/28/2005	Influenza A						35ul
FE2707 NW	4/5/2006	Influenza B	15ul					
FE0657 NW	5/1/2006	Influenza B				150		
042579 TS	9/8/2003	Adenovirus E4a	15ul					
F24954 NW	5/7/2007	Adenovirus B14		100ul				
F19392 NW		HSV			35ul			
F98585 NW		Enterovirus			35ul			
703667 TS		S. dysgalactiae			35ul			
		water					100ul	
* *S. dysgalactiae* subsp. *Equisimilis* (GenBank AY148430)								

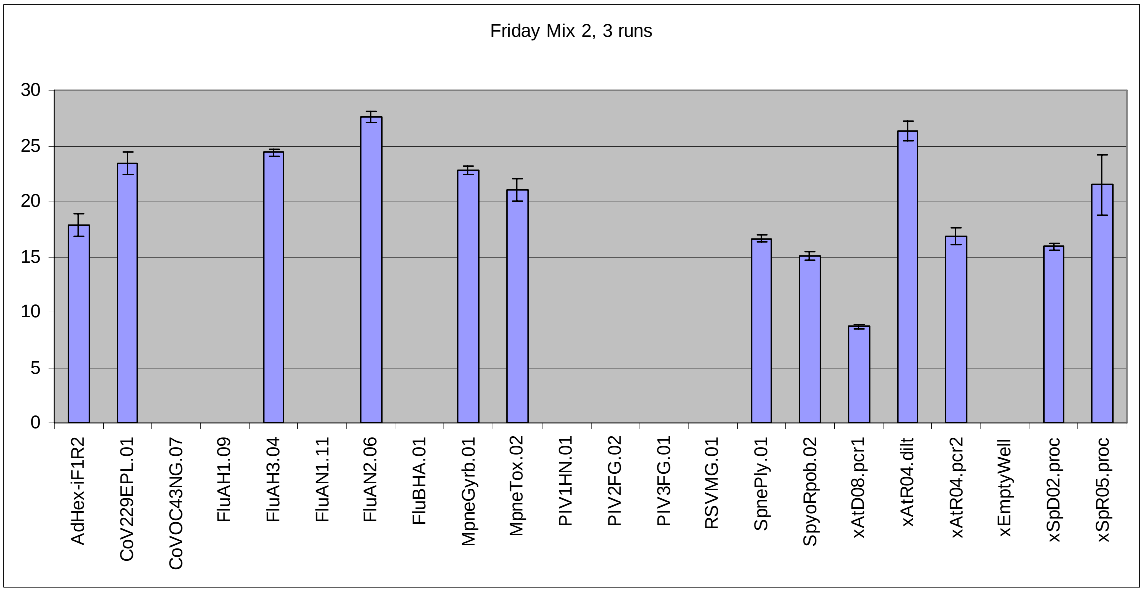
A mix of Influenza A (H3N2 and H1N1), Influenza B, Adenovirus B & E, Coronavirus 229E, Coronavirus OC43, RSV, PIV1, PIV2, PIV3, *M. pneumoniae*, *S. pyogenes*, and *S. pneumoniae* were extracted, amplified, and detected on the FilmArray. The first chart demonstrates the average cycle threshold per assay on the array with a mix of 7ul of each spiked agent at the following dilutions of the ATCC listed concentration: 1:10, 1:100, 1:1000, and 1:10000.



ATCC concentrations at 1X used in above dilution chart shown in table below:

Organism	CFU/CEID/TCID/ml
Influenza A H3N2	2.81E+07
Influenza A H1N1	8.89E+06
Influenza B	8.89E+06
Adenovirus B & E	8.89E+05
Coronavirus 229E	1.58E+05
Coronavirus OC43	2.45E+08
RSV	1.58E+07
PIV1	1.58E+08
PIV2	8.89E+06
PIV3	1.58E+09
M. pneumoniae	2.00E+06
S. pyogenes	9.80E+09
Strep. pneumoniae	8.00E+05

The chart below demonstrates the standard deviation of 3 runs of a mixture of agents at below concentrations (15ul each) that was kept blind to ITI prior to analysis.



The concentration and list of agents used in the above standard deviation chart are shown in the table below.

Positive Agents	**CEID/TCID/cfu/ml**
Influenza A H3N2	**2.16E+04**
Adenovirus B & E	**6.84E+02**
Coronavirus 229E	**1.22E+02**
M. pneumoniae	**1.54E+03**
S. pyogenes	**7.54E+06**
Strep. pneumoniae	**6.15E+02**
Negative Agents	
Staphylococcus aureus	**2.31E+04**
HSV-1	**2.16E+05**
Neisseria mucosa	**4.61E+06**
Hemophilus influenzae	**6.92E+04**

Assessment Recommendations; Benefits of the Film Array technology include low sample volume, automated sample preparation and analysis, using a closed system which reduces contamination risk, providing results at the sensitivity of a JBAIDS system, short time-to-result (<1hour), and a lower cost per reportable result (projected to be approx $4/result). The current reagent pouch costs $120 for 120 possible assays. The system in current format can test one sample for over 50 agents in less than one hour. The platform is small (8" x 11" x 17"), and there is no cold chain for the reagents. All manufacturing occurs in house, including reagents, buffers and assays. The company has a long history of working with the DoD, including combat developers and taking platforms through operational testing and fielding. In addition, ITI has developed numerous extraction protocols and passed government validation testing for extraction efficiency on all matrices (over 15 total). Sample matrices tested on the Film Array include tuna salad (washed in stomacher bag- this is an ITI developed protocol) and swabs (also washed). The software background of ITI includes vigorous military basic operator and end-user testing for ease of use as well as FDA certification. ITI uses USAMRIID signatures for BT assays, optimized to run at the same cycling parameters.

DNA sensitivity with bacillus spores and the bacillus anthracis assay demonstrated a sensitivity of 10^2 cfu/ml with 100ul volume added to the reagent pouch. This is a sensitivity of 10cfu/assay. ITI has demonstrated no interfering competition during nested PCR on the Film Array with the full panel of viruses from their respiratory virus cocktail being run at the same time with different concentrations, including Adenovirus, HRV, PIV1, PIV2, PIV3, RSV, Influenza B, BOCAvirus, HMPV, Influenza A (Matrix, H3, N2, H5 and N1) and the Coronaviruses 229E, NL63, OC43, HKU1, and SARS. The current array contains 120 wells, but competition was not reported by ITI until over 400 wells were present on an array. Clinical validation of the respiratory pouch is being conducted on 1200 pediatric samples. Beta instruments and respiratory virus pouches will be available mid-2007.

Although ITI has an extensive URI panel in-house and going through clinical validation testing (not FDA clinical trials), several important assays must be developed including Bordetella pertussis I and II, Enterovirus, Chlamydia pneumoniae, Influenza A, (H1, H7, H9) Streptococcus pneumoniae, Mycoplasma pneumoniae, Legionella "species", Haemophilus influenzae, Streptococcus pyogenes, Staphlococcus aureus, Neisseria meningitides "Y", and Moraxella catarrhalis.

In addition, although assays for all agents of EOS BT concern have been developed, lyophilized and optimized to run on the same cycling parameter, they have not all been run on the Film Array. Sensitivity for each assay must be worked out independently and simultaneously specifically on the Film Array.

Interpretive software was derived from the JBAIDS and LightCycler system software. ITI anticipates by mid-2007 they will be able to perform meta-analysis integrating results from controls and unknowns into a definitive call. The software in its current configuration is user friendly in a wizard format. A laptop will come with the system with complimentary software but not integrated the film array instrument. This software was not demonstrated at the ITI FilmArray pre-validation testing at the ADL. All data generated from the ADL FilmArray developmental testing is indicated is Appendix E.

AKONNI BIOSYSTEMS TECHNOLOGY EVALUATION

AKONNI BIOSYSTEMS TECHNOLOGY; The Akonni Biosystems' TruArray™ technology is a three-dimensional, medium density (50 – 200 feature) spotted hydrogel fluorescent microarray which can be used for both nucleic acid and protein analysis. Work under the current AF/SGR contract has focused on development of an integrated sample preparation protocol that can be used for both DNA and RNA without sample splitting, and development of a TruArray™ v 1.0 chip that can be used for upper respiratory pathogen detection based on genomic probe design with 5-7 targets per pathogen developed under a CRADA with Los Alamos National Laboratory. Sample preparation, RT-PCR and amplicon analysis using TruArray™ v 1.0 prototype chips are performed manually with an MJ Research PCR instrument and OEM array scanner/software from Aurora Photonics. The Akonni Biosystems Statement of Work and deliverables are included in Appendix I.

Akonni has made significant progress under the current AF/SGR contract and has submitted a follow-on BAA proposal (3 year, $ 9.7 million) to integrate and automate their TruArray™ chip technology for EOS application. Several issues were most apparent:

1. Workflow: The current manual TruArray™ workflow is too laborious and requires multiple instruments for sample preparation, amplification, hybridization and analysis.

2. FDA: There is no clear precedent (predicate device) or pathway for FDA approval of a TruArray™ device. Current research, development and manufacturing operations are not GLP or cGMP in their biotechnology incubator facility at Hood College.

3. Controls: Akonni has not addressed the issues of sample processing and analyte positive controls with their current TruArray™ protocol, or discussed these issues with FDA.

4. Nasal wash and throat swab matrices: Akonni has not tested actual nasal wash or throat swab samples. This was strongly recommended to augment their current work with spiked buffer samples.

5. Cross hybridization: Akonni presented initial results for 11 positive and 11 negative strains comparable to the EOS ACTD Pre-Validation Test Plan list. The initial results showed significant cross hybridization between pathogens for most positive strains tested, i.e. reactivity of fluA with fluA, fluB, and Strep. *pyogenes*. Conversely, most of the negative test strains also showed hybridization to targets for one or more pathogens. These results indicate specificity problems in the system.

After Action Report For 8 Jun 07 Site Visit; The site visit occurred on Fri, 8 Jun 07 from 1100 until approximately 1430 at the company's location in Frederick, MD. The USAF (AF) EOS Team was led by Maj Dempsey who was accompanied by Dr. Pete Andreotti, Dr. Cindy Orser, and Mr. Richard Baldwin. Dr. Chuck Daitch was present with his team as follows: Jennifer Reynolds, Eric Black, Phil Belgrader, Yvonne Linger, Lexi Bryant, Michelle Hulcher, Cindy Zimmerman, Nitu Thakore, and Darrell Chandler. Formal introductions were made and the meeting proceeded.

Maj Dempsey announced from the meeting outset that the intention of the AF was to assess the current contract with the EOS Program to include technology maturity and technical issues, and that continuation of the current contract would need to remain within scope of said contract with no further obligation of Government funding. He further offered that the AF EOS Team would be available to help guide the remaining milestones to achieve contract completion. Additionally, Maj Dempsey stated that since AFDW Contracting had formally requested full proposal of the new BAA pre-proposal (Dr. Daitch had the e-mail request of which Maj Dempsey made a copy), that minimal discussion of applicable R&D efforts could be entertained, but that the Government would not be obligated to any contractual agreements, and besides, that any future award would be based on Akonni's successful completion of the current contract's deliverables as a prerequisite.

Dr. Daitch provided a comprehensive history of the Akonni company as well as a detailed overview of the R&D leading to the current contract, as well as some proposed R&D for future award, if approved by the AF. At this point, the EOS Team (Drs. Andreotti and Orser) requested a detailed funding slide, which Dr. Daitch agreed to provide.

Following these discussions, the Akonni Team provided a tour of their R&D laboratories, including efforts toward achieving current contract completion, as well as some demonstration of potential technological advancements based on the new BAA proposal.

Following the R&D tour, Drs. Daitch and Chandler presented their data demonstrating their adherence to the current contract milestones. While it appeared that the company is on-track with the current contractual milestones, some significant technical issues emerged and were discussed, to include observed cross-hybridization, lack of analyte-specific positive controls, and use of appropriate clinical matrices (e.g., nasal washes and/or throat swabs). No clear solution was reached, but Drs. Andreotti and Orser provided comments and discussion.

Discussion proceeded to address the R&D schedule required to achieve the remaining milestones, to include parallel testing at the San Antonio Advanced Diagnostic Laboratory (ADL). First, however, an additional site visit/"milestone demonstration meeting" was proposed by the AF Team for the week of 23 Jul at which time performance of the pre-validation test (including multiplex testing of between 4 and all 11 mixed positive-panel pathogens) would occur. Subsequent to the 23 Jul meeting, then the second instrument would be setup at the ADL (in early August) for evaluation there.

Additional discussion involved a request by Mr. Baldwin to meet with Akonni personnel to address specific FDA issues. Also, the AF Team requested all briefing slides presented by Akonni during the meeting, of which Dr. Daitch agreed to provide NLT COB Mon, 11 Jun 07. Being no further business to discuss, the meeting adjourned approximately 1430 hrs.

AF/SGR agrees to continue to provide guidance towards successful completion of the current contract. It was recommended that we proceed to visit Akonni again on or about July 23, 2007, at which time Akonni could perform a Pre-Validation Test Plan demonstration, if Akonni believes they are ready to perform the demonstration. It is strongly recommended that Akonni perform the Pre-Validation demonstration for AF/SGR to evaluate as part of the BAA proposal review process.

After Action Report For 24 July 07 Site Visit; The meeting included two functions: 1). Presentations by Akonni of their technologies for viral and bacterial pathogen detection and identification using their TruPrep sample preparation technology and three dimensional gel droplet TruArray microarray system. Presentations were focused on progress under their current AF/SGR contract for multiplexed pathogen detection, development of their TruPrep sample preparation technology primarily funded by DTRA, and development of their new detection TruDiagnosis instrument. As requested, Akonni presented data for the TB and biothreat agent detection work with CDC, DTRA and USAMRIID which is outside the scope of their AF/SGR contract. 2.) A "start-to-finish" technology demonstration based on the provided protocol. The technology demonstration included start-to-finish testing of an adenovirus sample (10^4 PFU/ml aliquot on Dacron swab), testing a mixture of previously isolated adenovirus DNA, coronavirus RNA, *Strep. pyogenes* DNA and *Myco. pneumo* DNA (gram negative), and testing a no template negative control (NTC) sample. The demonstration was completed within 4 hours. The demonstration successfully identified the adenovirus, mixture of adenovirus, coronavirus, *Strep* and *Myco* nucleic acids, and was negative for the NTC.

Data was also presented for additional studies using single and mixed pathogen preparations for 11 pathogens identified by AF/SGR as "positive panel" agents and 11 microorganisms identified by AF/SGR and "negative panel" agents. The reader is referred to the electronic presentations provided by Akonni. Key findings for presented data include:

1. Akonni was able to successfully identify up to 7 agents in a mixture using previously isolated nucleic acids.
2. Akonni was not able to successfully test 4 agents (adenovirus, coronavirus, *Strep* and *Myco*) in a "start-to-finish" test with intact organisms. They were, however, successful starting with isolated nucleic acids. The reason for this difference is not understood. In this effort, Akonni 'failed" the basic 4-pathogen model test used previously for most pMD studies. Akonni also has not been able to successfully complete the AF/SGR staffed "pre-validation" test plan which is an exit criterion for their current AF/SGR contract.
3. Akonni has significant pre-clinical data for the use of their system to detect/diagnose TB and drug resistant TB in sputum samples. Akonni has an active collaboration with leading TB investigators at CDC for this effort, and plans their first 510(k) for this application.
4. Akonni has significant data for detection of BT/BW agents using their microarray system. This work is based on their DTRA funded effort for development of a universal sample preparation protocol and use for BT/BW agent testing.

At this stage, it is recommended to wait until their current AF/SGR contract has been completed, and then re-evaluate the status of the technology with a close-out presentation to the EOS scientific team. Consideration should be given to the possible use of their technology for TB and BT/BW agent detection for the EOS ACTD.

CEPHEID TECHNOLOGY EVALUATION

Cepheid Technology Review; The technology evaluation performed for the Cepheid GeneXpert system was performed from March 21-April 6, 2007. The EOS ACTD technology evaluation team sought to apply a standardized process for each company and technology under consideration. For the Cepheid GeneXpert system, the process included electronic exchange of information with Mr. William Goodwin and Dr. Peter Dailey of Cepheid. Meetings were held in Washington, D.C. with Mr. Goodwin, Dr. Emily Winn-Deen, VP for Strategic Planning and Business Development, and Dr. Michele Schoonmaker, Director for Government Affairs. An approximately 6 hour meeting and site visit to Cepheid was held March 30, 2007 attended by Dr. Peter Dailey, Vice President for Research and Development, Dr. Alan Wortman, Senior Director for Development, Dr. Chris Wilkins, Director for US Sales and Marketing, Francisco Dias Lourenco, Director for Biothreat Operations, Humberto Reyes, Executive Vice President for Operations, and Dr. Rick Rodgers, Development Director. This meeting and site visit was attended by Dr. Peter Andreotti, Dr. Lisa Lott, and Dr. Peter Estacio.

Cepheid is an RT-PCR molecular diagnostic company focused on providing FDA approved molecular diagnostic devices. The company is committed to providing FDA cleared molecular diagnostic assays for the GeneXpert platform and has requisite design control processing and QSR processing in place. The company has two main platforms: 1.) The SmartCycler and 2.) The GeneXpert. This technology evaluation is for the GeneXpert system, as detailed in Appendix J. Both the SmartCycler and GeneXpert use the same “I-core” technology to perform RT-PCR derived from research and development in the DARPA Advanced Diagnostics program in the 1998-2002 time period. The GeneXpert is the more advanced, scalable technology platform that integrates sample preparation and RT-PCR in a single use cartridge. The key feature of the GeneXpert system is the easy-to-use, rapid work flow with minimal (less than 5 minutes) “hands-on” time. The company advertises “total integration” and “sample in, answer out” that includes adding 0.1-5.0ml of sample to the Gx cartridge, inserting the cartridge into the Gx, and then starting the automated sample preparation and RT-PCR in the cartridge and obtaining results in 3-60 minutes with an interfaced PC. For some cartridges, 1-2 liquid reagents need to be added to the cartridge with unit dose dropper bottles. The Gx system is appreciated for its simple, rapid, and easy-to-use integrated work flow. However, the Gx is a closed system not amenable to end user modifications since all of the key sample preparation and assay reagents are contained in the closed cartridge, and the cartridges are relatively expensive in the range of $27-$50 per cartridge.

A key issue for the Gx has been the level of multiplexing that could be achieved. The original Gx cartridge concept was limited to 4 colors to give two targets and two controls per cartridge. In 2005, the Gx was evaluated for the EOS ACTD ad rejected primarily because of the limited multiplexing capability, despite the clear advantages for a rapid, integrated and easy-to-use system. These discussions occurred with Dr. Bill McMillan who was VP, Research and Development at Cepheid. More recently, Cepheid has embarked upon at least two research and development programs to develop multiplexing capability.

The Gx platform should be able to meet level 5 and 3 EOS ACTD technical requirements, but not the level 5 or 3 cost requirements, in the 2007-2011 time period,

assuming the new 6-color multiplex Gx cartridge technology works as planned. This would provide a “multiplex” system in which multiple cartridges could be analyzed simultaneously (the Gx instrument can analyze 10-4 cartridges simultaneously) to provide 16 pathogen assays plus controls within a 60 minute TAT. There would be some loss of sensitivity due to splitting of the sample into multiple cartridges. However, the level of sensitivity that can be achieved with the Gx (50 copies/cartridge) should be sufficient to compensate for this. The probability that the 6-color Gx cartridge technology will work is very high. The 36 multiplex cartridge technology is less certain.

The 6-color multiplex Gx technology should be available for initial evaluation in late 2008 or early 2009 with the CDC influenza panel. This is a deliverable for the HHS/CDC grant to develop the Gx for POC testing with the CDC influenza panel for AI surveillance in 2009. Overall, development of the Gx platform with this “multiple 6-color cartridge strategy” would require up to four years to complete for the proposed EOS ACTD upper respiratory panel, or commercial diagnostic equivalent as originally proposed by Dr. Pete Dailey when he was at Roche. This panel would likely include 4 cartridges:

1.) Influenza Panel (cartridge): Flu A, H1, H3, H5 and Flu B
2.) CAP Panel (cartridge): Strep pneumoniae, C. pneumoniae, M. pneumoniae and L. pneumophila
3.) Respiratory Panel (cartridge): Adenovirus (pan), PIV 1,2 and 3, RSV A/B, HMPV
4.) Bacterial Panel (cartridge): B pertussis/parapertussis, others TBD

Additional cartridges could also be developed for BT agents to complement the existing 3-agent BT panel marketed by Cepheid and used by the USPS and State Department for environmental testing. It is important to note that the existing 3-agent BT panel for environmental testing is the only assay panel that would be available to meet EOS ACTD requirements in June 2008. All other assays would require new or additional research and development for the Gx cartridge, which would require at lease 12-18 months, even for the ASR assays currently available for the Cepheid SmartCycler.

The Gx platform is an attractive molecular diagnostic RT-PCR system because of its integrated, rapid and easy-to-use workflow which combines sample preparation and RT-PCR into a single cartridge that can be used to test nasal wash, swabs and blood with a small footprint system. There is also a commitment by Cepheid to pursue FDA-IVD and CE-IVD products as its core business. However, while the Gx instrument per se is relatively inexpensive at under $100k for the 1-4 cartridge configurations, the cartridges are relatively expensive compared to other RT-CPR systems, and the system is closed to modification by the customer.

PROPOSED WORKPLAN BETWEEN CEPHEID AND USAF-EOS

Goals of Agreement; Cepheid has been contracted by the Centers for Disease Control and Prevention (CDC) and the U.S. Department of Health and Human Services, to develop an in vitro diagnostic system that will detect seasonal influenza and differentiate seasonal influenza from potentially pandemic highly pathogenic influenza H5N1. The objective of the work between USAF-EOS and Cepheid will be to validate early and advanced prototypes of the IVD system using specimens isolated from humans with influenza-like illness.

GeneXpert Technology; The Xpert Flu assay will be a real-time multiplex RT-PCR assay designed to detect and differentiate Flu A, Flu B, Flu A/H1, FluA/H3 and Flu A/H5. The multiplex assay will also include primers and probe for detection of a sample processing/inhibition control that will be included in every run. All assay components and the SPC/IC template will be dried down into beads and loaded into GeneXpert cartridges. The cartridges will also contain all the reagents required to perform the extraction and purification of nucleic acid from the specimens. Only specimen will need to be added to the cartridge. Sample types will be nasopharyngeal and/or throat swabs in viral transport medium. An aliquot (approximately 200µL) of the VTM is transferred to the cartridge using a transfer pipette. Cartridges will be run on the GeneXpert Diagnostics system. Time to result will be approximately 30 minutes.

Program Testing; The duration of the program will be up to 20 months, including participation in the alpha and beta trials as is appropriate. Beta Trial testing will include an evaluation of the performance of the Xpert Flu product with human specimens. The beta trial is scheduled for November/December 2007. Specimens will consist of prospectively collected throat and nasopharyngeal swabs and nasal aspirates from patients presenting with influenza-like illness. The number of specimens required will be determined by the Cepheid Clinical group. Confirmation of the results will be performed by traditional gold standard viral culture and subtyping of all the prospectively collected specimens. Isolation of RNA from specimens giving discrepant results in the trial will also be performed followed by RT-PCR and sequencing. Beta testing requires accurate collection and timely reporting of the results of the testing and a final report at the conclusion of the trial. Feedback will be provided on the positive and negative attributes of the Xpert Flu test. Archival or prospective leftover human specimens will be supplied to Cepheid in order for us to evaluate the performance of prototypes of the Xpert Flu product. Xpert flu results will be compared with data gathered when specimens were originally tested.

Cepheid will provide:

1. Instrument for the beat trial collaboration: 1 six-color GeneXpert instruments for the duration of the agreement on Material Transfer Agreement. The instruments would remain the property of Cepheid, unless provided for under grant or some other external funding source.

2. Supply of all cartridges for running the tests.

3. Supply of collection devices for taking, transporting and storage of specimens.

4. Devices, instructions and protocols for running the tests.

5. Technical support for use of the GeneXpert®.

6. Reimbursement of costs for participation of USAF-EOS in the project.

Documentation; USAF-EOS and Cepheid collaborators will ensure that all legal issues and documentation requirements are brought to the attention of Cepheid and ensure that all requirements are met prior to the start of the trials. All documentation for relevant tests and protocols that will be used during prosecution of tasks will be provided to both parties. Information regarding validations of the tests should also be supplied.

NANOGEN TECHNOLOGY LEVEL REVIEW

Technology Readiness Level (TRL) evaluation; The technology evaluation was performed for the Nanogen NC 400 Nanochip Microelectronic Array as described in the Nanogen White Paper (Appendix K) with the 9 component matrix utilized previously by AF/SGR EOS ACTD for evaluation of Roche, Applied Biosystems, NRL, Affymetrix and Combimatrix RT-PCR or microarray platforms for medical diagnostics. TRL criteria for Medical Devices are based on Joint Program Executive Office for Chemical and Biological Defense Assigning Technology Readiness Levels criteria Prepared for the JPEO-CBD by The National Assessment Group 5 August 2004.

The evaluation was initially performed by Dr. Michael Dempsey (AF/SGR) with TRL values proposed by Nanogen. Additional information was obtained for TRL evaluation during a site visit demonstration and face-to-face discussions between Dr. Peter Andreotti (AF/SGR EOS ACTD), CDR Kurt Henry, Dr. Dalibor Hodko (Director of Advanced Technology, Nanogen), Dr. Graham Lidgard (Senior Vice President, Research and Development, Nanogen), Mr. Robert Bush (Vice President, Sales, Nanogen), and Mr. David Ludvigson (President, COO, Nanogen) on 8 February, 2007, at Nanogen offices in San Diego, CA.

The Nanogen NC 400 Nanochip Microelectronic Array is based on an electronically controlled 100 - 400 individually programmable site microchip embedded in a microfluidic cassette. The system utilizes a benchtop reader with proprietary software to quantify fluorescent reporter nucleic acid or protein probes. The system is currently being evaluated by scientists at BDRD for BW applications. The NC400 Nanochip Microelectronic Array requires third party instrumentation for sample nucleic acid (NA) isolation and purification, and third party instrumentation for isolated/purified NA amplification prior to quantification of the amplified NA on the Nanochip. The current commercial system configuration uses the Roche MagnaPure Compact for NA isolation/purification, and the ABI 7500 thermocycler for NA amplification.

Reader Instrument; The NC400 reader instrument is in the TRL 8 range as the instrument is QSR compliant (manufactured by Hitachi), has received CE mark, is manufactured under ISO. TRL levels for the 9 component matrixes are under FDA review or pre-IDE for 510(k) for a number of assays including cystic fibrosis, hemochromatosis and a 7 target respiratory viral panel that are already being sold in Europe under CE regulations.

Reader Instrument Software; The NC 400 reader instrument software is in the TRL 8 range as the instrument with embedded software is QSR compliant (manufactured by Hitachi), has received CE mark, is manufactured under ISO 9000, and is under FDA review or pre-IDE for 510(k) for a number of assays including cystic fibrosis, hemochromatosis and a 7 target respiratory viral panel that are already being sold in Europe under CE regulations. A proprietary closed system diagnostic software package for the instrument is under development.

Core Diagnostic Assay Technology; The core diagnostic assay technology is in the TRL 8 range. More than 200 assays have been developed for the NC400 technology.

Assays are commercially available for diagnostic use in Europe under CE label. ASR assays are available in the U.S. Additional assays are in 510(k) process with FDA. These include a 7 target respiratory viral panel that is already being sold in Europe under CE regulations, and a 10 target (7 bacteria and 3 viruses) panel under development in the U.S.

Diagnostic Assay Format; The diagnostic assay format is in the TRL 8 range. This component has been included in the EOS ACTD TRL review process to evaluate platform assay format to perform multiplexed assays for 20 – 100 pathogens in ≤ 4 hours in order to meet ACTD exit criteria. The NC400 can test up to 400 targets including controls simultaneously, or in sequential patterns (i.e. the chip can be re-used to sequentially test unused pads or sites on the chip). Also, multiple samples with controls can be tested on the same chip using sequential protocols. The current total assay time is 4-5 hours which includes nucleic acid isolation/purification, NA amplification and measurement/reporting with the Nanochip 400.

Upper Respiratory Pathogens Validated; This component has been included in the EOS ACTD TRL review process to evaluate platform capability to perform assays for upper respiratory pathogens causing ILI symptoms. Nanogen currently markets a 7 target respiratory viral panel in Europe, is developing a 10 target upper respiratory panel, and is one of four companies that received a HHS/CDC contract to develop a test system for near point-of-care influenza panel testing (system available in 2008). The current state of upper respiratory pathogen assay development and validation is consistent with TRL 7.

Sample Preparation Instrument; The Nanochip NC 400 requires third party instrumentation for nucleic acid isolation/purification and amplification before the same can be quantified on the Nanochip. The current commercial system utilizes the Roche MagnaPure Compact and the ABI 7500 for isolation/purification and amplification, respectively. Both of these third party instruments are research use only. In accord with previous TRL evaluations for RT-PCR and microarray platforms used for public health and homebrew clinical applications, this is consistent with a TRL 6.

Blood Sample Preparation; This component has been included in the EOS ACTD TRL review process to evaluate the technology used to prepare blood sample nucleic acids (including isolation, purification and amplification) for RT-PCR or microarray testing. The Nanochip NC 400 requires third party instrumentation for nucleic acid isolation/purification and amplification before the sample can be quantified on the Nanochip. The current commercial system utilizes the Roche MagnaPure Compact and the ABI 7500 for these components. In accord with previous TRL evaluations for RT-PCR and microarray platforms used for public health and homebrew clinical applications, this is consistent with a TRL 6.

Upper Respiratory Sample Preparation; This component has been included in the EOS ACTD TRL review process to evaluate the technology used to prepare upper respiratory sample (nasal wash, throat swab) nucleic acids (including isolation, purification and amplification) for RT-PCR or microarray testing. The Nanochip NC 400

requires third party instrumentation for nucleic acid isolation/purification and amplification before the sample can be quantified on the Nanochip. The current commercial system utilizes the Roche MagnaPure Compact and the ABI 7500 for these components. In accord with previous TRL evaluations for RT-PCR and microarray platforms used for public health and homebrew clinical applications, this is consistent with a TRL 6.

Complete System Integration; This component has been included in the EOS ACTD TRL review process to evaluate the overall integration of sample preparation, diagnostic assay, measurement (reader) and reporting (software) technologies. A TRL level of 6 is proposed based on the limitation that the overall NC400 system requires two third party components for NA sample isolation/purification and amplification.

Additional Comments; The Nanochip Microelectronic Array is a mature technology used worldwide for advanced microarray applications for both nucleic acids and proteins, and is marketed in Europe under CE mark for diagnostic applications. The NC 400 system is a flexible platform to quantify and qualitatively analyze multiplexed amplicon samples. However, it does require front-end nucleic acid sample isolation/purification and, more importantly, it requires a front-end nucleic acid amplification (PCR) step using third party instruments such as the ABI 7500. Instead of performing both amplification and quantification simultaneously (real-time PCR such as Taqman), the Nanogen system first performs a separate PCR step and then relatively rapidly (60 minutes) quantifies and analyzes the PCR amplicon products on the Nanochip.

MESOSCALE TECHNOLOGY LEVEL REVIEW

Technology Readiness Level Evaluation; This report summarizes the results of a technology evaluation of Meso Scale Diagnostics, LLC (MSD) that was conducted to determine the suitability of MSD's technologies for inclusion in the Epidemic Outbreak Surveillance Advanced Concept Technology Demonstration (EOS-ACTD) Program.

The evaluation presented in this document is based on the following: 1) A technology readiness level (TRL) evaluation previously performed by Dr. Peter Andreotti that assigned a TRL 5-6 to MSD PR2 Multi-Array Electrochemiluminescence (ECL) Immunoassay System; 2) Literature previously provided by MSD for the initial TRL evaluation including a white paper submitted by MSD and 3) A site visit on 27 March 2007 to MSD offices in Gaithersburg, MD, where Dr. Peter Andreotti (AF/SGR EOS ACTD), LT Franca Jones (NMRC), and Mr. Richard Baldwin (AF/SGR EOS ACTD) met face-to-face with Dr. George Sigal (Director of Research, MSD) and Dr. Charles Clinton (Senior Scientist, MSD) to discuss current technology advances.

The MSD Platform is a multiplexed microplate immunoassay system that utilizes a benchtop instrument to quantify electrochemiluminescent (ECL) ruthenium labeled reporter antibodies. There are four MSD instrument platforms which utilize the same ECL technology. MSD currently markets two (Sector™ Imager and Sector™ PR) commercial-off-the-shelf (COTS) instruments that both have the CE mark and are for research use only (RUO). The Sector™ Imager Instrument can run a 24-, 96-, or 384-well multi-spot (assay) plate and Dr. Clinton noted that the 96-well plate with 4, 7, or 10 spots per well is MSD's main focus. The Sector™ PR also runs 96-well plates but has limited multiplexing capability. Approximately 400 COTS instruments have been sold to research laboratories throughout the world, the majority of which are pharmaceutical companies that perform drug discovery and/or biomarker measurements during clinical trials. MSD manufactures approximately 120,000 plates per year containing assays for over 100 biomarkers used in life science research to include assays for clinical markers, cytokines and chemokines, and markers of cell signaling pathways. Detection limits in multiplexed immunoassays for protein analytes can reach as low as 100,000-200,000 molecules with a dynamic range of 104-105. To service its growing customer base, MSD built a manufacturing facility with robotics printers for rapidly printing arrays in its plates.

MSD's two non-COTS platforms are significantly smaller and include the PR2 and the cartridge reader. The PR2 was developed to support biodefense applications (funded by Defense Threat Reduction Agency) and the Cartridge Reader is being developed as a point of care diagnostic for detection of pandemic influenza (funded by Centers for Disease Control) and bioterrorism (BT) agents in clinical samples (funded by National Institutes of Health).

The PR2 is a small, lightweight instrument capable of multiplex detection up to 25 spots per well of a 96-well plate. Each plate takes approximately 1 hour from sample receipt to result. Because these systems do not employ nucleic acid amplification, little (in the case of nasal and throat swabs) or no (other clinical and environmental matrices) sample processing is required. The PR2 has been evaluated by several laboratories including the John's Hopkins Applied Physics Laboratory (JHU-APL) for the U.S. Army Research, Development, and Engineering Command (REDCOM), Edgewood Chemical,

Biological Center (ECBC), Naval Medical Research Center (NMRC), and at a Technology Readiness Evaluation at Dugway Proving Grounds in 2006. JHU-APL assigned the PR2 a TRL of 6 for its ability to detect BT agents. Both ECBC and NMRC have given the PR2 high marks for BT agent detection. Dr. Andreotti also gave the PR2 a TRL of 5-6 based on MSD's limited work with non-BT respiratory agents

Although not currently available for external testing and evaluation, the Cartridge Reader is a promising new technology being developed by MSD. Like the pMD cartridge, being developed by Applied Biosystems, the cartridge will allow for up to 16-20 spots per cartridge per sample and will take only 15 minutes to read. MSD has a 2-3 year timeline for developing the cartridge reader to do point of care diagnostics for pandemic influenza and also has funding to take this technology through FDA approval. In March 2006, the Cartridge Reader was evaluated by JHU-APL as part of the Joint Biological Agent Identification System (JBAIDS) Block III Tech Watch. The Cartridge Reader was assessed to be at TRL 3 and was expected to be at TRL 7 by December 2008. Dr. Andreotti estimated the Cartridge Reader to currently be at TRL of 4. The Cartridge has an internal extraction chamber for swabs (nasal/throat) or liquids (blood/urine).

MSD Technology Evaluation; Developmental technologies from Meso Scale are illustrated in Appendix L titled New Developments in Electrochemiluminescence (ECL) Assays. MSD's currently available assays include 10 BT agents, Flu A and B nucleoprotein and Flu A H5 hemagglutinin, and 4 non-flu respiratory pathogens. MSD is also funded by the CDC to develop assays for H1, H3, H5, H7, and H9. It is expected that these assays will be developed by June 2008. Assays for other respiratory pathogens and any other BT agents selected by the Scientific Advisory Board could be developed to meet ACTD expectations in the future with approximately 1 year of additional funding.

MSD has experience with multiple sample types including nasal and throat swabs, blood, including whole blood, serum, and plasma, and environmental samples, including samples derived from aerosol collection systems as well as dry filter units. In all cases there is either limited, in the case of nasal and throat swabs, or no sample preparation prior to introducing the sample into the test well. For analysis of clinical swabs, the PR2 instrument may require an offline swab extraction step. A swab would be used to collect the sample and then the swab would be placed into a test tube that is filled with a buffer solution. The swab is then swirled in the buffer solution and then squeezed against the side of the tube. The resulting buffer solution would then pipetted into a well of the plate. For the cartridge reader instrument, automated swab extraction is integrated into the cartridge itself.

Performance metrics for the PR2 are shown below. The table gives a comparison of detection limits (concentration that gives a signal 2.5 standard deviations above background) determined by internal testing at MSD and external testing at ECBC for one lot of plates designed with 10-target multiplexing per well. The major cause of the differences between results at the two facilities is differences in the inactivated antigen preparations used in the studies. Turnaround time for these assays was between 1-1.5 hours.

	Internal Results	External ECBC Results
BWA	**Det. Limit**	**Det. Limit (Ave of 3 Experiments)**
BA	4000 CFU/mL	94±34 CFU/mL
BotA	1.6 pg/mL	8±2.9 pg/mL
Brucella	9200 CFU/mL	294±136 CFU/mL
FT	140 CFU/mL	187±30 CFU/mL
Q Fever	490,000 CFU/mL	43,000±19,000 CFU/mL
Ricin	1.4 pg/mL	1.0±0.3 pg/mL
SEB	1.0 pg/mL	1.4±0.8 pg/mL
Vaccinia	120,000 PFU/mL	6,600±640 PFU/mL
VEE	340,000 PFU/mL	480,000±23,000 PFU/mL
YP	10 CFU/mL	25±13 CFU/mL

The table below shows internally generated detection limits for MSD's flu assays and for a number of other respiratory infection (RI) pathogens.

RI Category	Assay	Detection Limit
Flu	Flu A (NP)	120,000 virus particles/mL
	Flu B (NP)	220,000 virus particles/mL
	Flu A (H5)	99,000 virus particles/mL
Other RI	RSV	25,000 virus particles/mL
	Staph aureus	7,200 CFU/mL (MRSA strain)
	Strep pneumoniae	4 CFU/mL (Type 1)
	Strep pyogenes	0.5 CFU/mL

Specificity testing of the BT agent panel was carried out with 32 closely related agents or interferents. NMRC also tested the BT agent panel against the Critical Reagent Program's interference panel. Both studies demonstrated high specificity and no effect from interferents, except for the VEE assay, for which MSD is optimizing.

Current and Future ACTD Expectations; It is expected that the PR2 will easily meet Level 3 exit criteria by June 2008. Level 3 criteria is defined as testing 20 pathogens under 4 hours by one technician, and a cost and size requirement of $75 per test and 15ft2, respectively. The 96-well plates for the PR2 can be configured to assay for 25 agents per-well in approximately 1 hour. The current footprint of the PR2 is less than 3 ft2 including the laptop. Current assay costs range from $2-$4 per sample. The PR2 was designed to be rugged, is packaged in a pelican case, and a tough book computer can be provided. The PR2 plates are packaged in hermitically sealed foil pouches with desiccant packs to protect them, prior to use, from ambient humidity. Our current specification is greater than one year stability for the plates at 4 C in their original packaging. Stability studies at room temperature are ongoing. The PR2 plates have been environmentally hardened to allow the plates once removed from their packaging, to withstand one month exposure at room temperature and 100% relative humidity.

In the future, it is expected that all assays developed on the PR2 are transitioned to the cartridge reader. Assay performance should be unchanged. The cartridge reader

has a footprint about half the size of the PR2, has an internal swab extraction component, and is the device that MSD plans to take through the FDA clearance process. Although the cartridge system has reduced throughput, it still maintains a good level of multiplexing (16-20 agents per cartridge) for one sample and TAT is 15 minutes.

Consumables and instruments are manufactured in a highly controlled environment at MSD that includes rigorous process documentation and control. Plate components and reagents are sourced externally. All assembly and reagent coating processes are performed in-house at MSD in a class 100,000 clean room using highly-automated, high-volume manufacturing equipment. The consumables manufacturing processes is backed by extensive in-process quality control including the use of machine vision systems for automated optical inspection.

MSD assembles and tests its commercial instrumentation in-house. Individual custom and COTS components are sourced externally. MSD maintains rigorous quality control over manufactured instrumentation. Under currently funded programs in the clinical diagnostic and biothreat areas, MSD is in the process of implementing ISO9000:2000 and cGMP/QSR quality systems. These manufacturing controls will be implemented over the course of the next 3 years.

By June 2008 it is expected that MSD will only commercialize their assays for RUO. They are funded by the CDC to take the influenza panel and the cartridge reader for FDA clearance in 2-3 years.

MSD identifies multiple sources for components wherever possible. This approach is complemented by putting in place supplier agreements for critical components. Finally, MSD inspects all incoming raw materials to ensure materials comply with specifications. MSD uses antibodies from the Critical Reagents Program and Tetracore for designing its BT agent plates and CDC antibodies for the influenza panel.

MSD has a contract funded by the CDC for developing POC diagnostics in conjunction with the Cartridge Reader for detecting pandemic flu strains. This 3 year program includes taking the diagnostic product through the FDA clearance process. MSD hopes to piggyback additional assays for respiratory agents onto the process for getting the pandemic flu assays cleared. Obtaining FDA clearance is in line with MSD’s internal plans for applying its PR2 laboratory instrument and Cartridge Reader POC instrument to clinical applications. MSD’s ability to make this commitment for the PR2 will depend on the number and identity of the assays required by the AF and the intended use of the assays.

MAGNABIOSCIENCES, LLC

Technology Readiness Level Review of the Magnetic Assay Platform; Technology Readiness Level (TRL) evaluation was performed for the MagnaBioSciences, LLC Magnetic Assay Platform using the 9 component matrix utilized previously by AF/SGR EOS ACTD for evaluation of Roche, Applied Biosystems, NRL, Affymetrix and Combimatrix RT-PCR or microarray platforms for medical diagnostics. TRL criteria for Medical Devices are based on Joint Program Executive Office for Chemical and Biological Defense Assigning Technology Readiness Levels criteria Prepared for the JPEO-CBD by The National Assessment Group 5 August 2004.

The evaluation was initially performed by Dr. Lisa Lott (ADL, Lackland AFB) using information obtained from MagnaBioSciences and telephonic discussions with personnel at MagnaBioSciences. Additional information was obtained for TRL evaluation during a site visit demonstration and face-to-face discussions between Dr. Peter Andreotti (AF/SGR EOS ACTD), CDR Kurt Henry, Ronald LaBorde (Technical Director, Biotechnology Division, MagnaBioSciences), David M. Pratt (Biotechnology Project Manager, MagnaBioSciences), and Dr. Stefano Spagna (Director of Engineering, MagnaBioSciences) on 8 February, 2007, at MagnaBioSciences offices in San Diego, CA.

The MagnaBioSciences Magnetic Assay Platform is a lateral flow, handheld immunoassay system which utilizes a small benchtop or handheld instrument to quantify magnetic nanoparticle reporter antibodies. The system is currently being evaluated by scientists at BDRD for BW applications. The platform is reported to give 10 - 1,000 times increase in sensitivity compared to non-magnetic lateral flow, handheld immunoassay.

Reader Instrument: The benchtop reader instrument is in the TRL 7-8 range as the instrument has received CE mark, is manufactured under ISO 9000, and is under FDA review or pre-IDE for 510(k) Chlamydia, Gonorrhea, HCG and Troponin-1 assays. The prototype handheld instrument demonstrated at MagnaBioSciences is estimated to be in the TRL 4-5 range.

Reader Instrument Software: The benchtop reader instrument software is in the TRL 7-8 range as the instrument with embedded software has received CE mark, is manufactured under ISO 9000, and is under FDA review or pre-IDE for 510(k) Chlamydia, Gonorrhea, HCG and Troponin-1 assays.

Core Diagnostic Assay Technology: Lateral flow, handheld assay systems have been FDA cleared for numerous applications. The core diagnostic assay technology for this magnetic nanoparticle reporter based system is in the TRL 7-8 range given that Chlamydia, Gonorrhea, HCG and Troponin-1 assays are under FDA review or pre-IDE for 510(k).

Diagnostic Assay Format: This component has been included in the EOS ACTD TRL review process to evaluate platform assay format to perform multiplexed assays for

20 – 100 pathogens in $\leq$ 4 hours in order to meet ACTD exit criteria. The MagnaBioSciences Magnetic Assay Platform lateral flow device (cassette) can perform up to 3 assays simultaneously on a single cassette. This may be increased to 7 assays per cassette with further development. The multiplexed format for the MagnaBioSciences Magnetic Assay Platform can be approached differently. Each cassette required a 10 – 30 minute assay time and 10 – 15 second measurement read time after the cassette is inserted into the benchtop (or handheld) reader. Hence, a single sample could be distributed over multiple 1 – 7 assay cassettes which could be measured sequentially (10-15 seconds/cassette) after a 10-30 minute assay (incubation) period. The magnetic readout is stable for an extended time. Based on this sequential multiplex format, a TRL level of 5-6 is estimated. A TRL level of 7-8 is commensurate with a single assay or low multiplex (1-3 assays for cassette).

Upper Respiratory Pathogens Validated: This component has been included in the EOS ACTD TRL review process to evaluate platform capability to perform assays for upper respiratory pathogens causing ILI symptoms. The MagnaBioSciences has been working primarily with BDRD on environmental testing of BW agents (Y. pestis, VEE, SEB etc) in addition to 510(k) applications for Chlamydia, Gonorrhea, HCG and Troponin-1 assays as noted above. Preliminary proof of concept studies have been performed for Flu A and Flu B (results provided by MagnaBioSciences), Dengue, *E. coli* (food testing) HIV, p24, malaria, THC (saliva), syphilis. This component for upper respiratory pathogens as defined by AF/SGR Tier 1 and 2 pathogens is the least mature for the MagnaBioSciences platform with a TRL of 4 – 5.

MagnaBioSciences is in the development of MICT Flu A/B assay devices for influenza A and influenza B antigen detection in nasal and throat swab samples. Current device prototypes can detect low level of influenza viral culture that can not be detected by other commercialized rapid test kits (Appendix M).

Sample Preparation Instrument: No sample preparation instrumentation is required and thus this component is not applicable.

Blood Sample Preparation: This component has been included in the EOS ACTD TRL review process to evaluate the technology used to prepare blood sample nucleic acids (including isolation, purification and amplification) for RT-PCR or microarray testing. This is not applicable for the MagnaBioSciences platform.

Upper Respiratory Sample Preparation: This component has been included in the EOS ACTD TRL review process to evaluate the technology used to prepare upper respiratory sample (nasal wash, throat swab) nucleic acids (including isolation, purification and amplification) for RT-PCR or microarray testing. This is not applicable for the MagnaBioSciences platform.

Complete System Integration: This component has been included in the EOS ACTD TRL review process to evaluate the overall integration of sample preparation, diagnostic assay, measurement (reader) and reporting (software) technologies. A TRL

level of 4 – 5 is proposed based primarily on the limited information available for Upper Respiratory Pathogens Validated as described above.

Additional Comments: The MagnaBioSciences Magnetic Assay Platform is mature technology after approximately 9 years of development, and is an “enhanced” version of well established lateral flow immunoassay technology that offers increased sensitivity, and stable quantitative results compared to other lateral flow methods. Immunoassays are generally regarded to be overall less sensitive[1] compared to genomic assays, and the quality of assays are primarily dependent on the availability of specific, high affinity antibodies.

ARIZONA STATE UNIVERSITY TECHNOLOGY READINESS LEVEL REVIEW FOR THE PORTABLE BIOLOGICAL ASSISTANCE CAPABILITY FOR PROTECTING A POPULATION (PBA)

A white paper describing the PBA technology capability is attached (Appendix N). The table below demonstrates an overall TRL of 2 due to lack of respiratory pathogens developed and a lack of overall system integration. The reader instrument was given a TRL level of 7 due to the advanced, commercially available detection instrument with validated core diagnostic assays.

Component	TRL	TRL Explanation	Comments
Reader Instrument Benchtop	7	Benchtop detection instrument commercially available as beta-test product through HTG / SDD;	Retail price range $25,000 to $35,000 Manual bioassay required
Reader Instrument Portable	4	Opto-electronic components for detector identified; Microfluidic actuation for automated bioassay demonstrated; all electronic controller components designed and tested	PCB design for compact camera-board required (in-house design capacity existing); integration in progress; Automated bioassay cartridge designed and prototyped
PBA Instrument Software - data analysis (benchtop)	4	Software architecture of PBA compatible with commercial imaging system; Pseudo-code defined, software algorithm for application to be developed.	Optimization for PBA microarray configuration required (due to be completed in 6 months)
PBA Instrument data acquisition Software	4	Actuator/sensor labview control completed, transitioning to micro-chip based programming in progress	OLED display function demonstrated, driver under development. Low cost LCD module identified for first prototype
Core Diagnostic Assay Technology	6	qNPA assay for micro-titer plate format validated (manual assay); The assay used in commercial drug discovery with multiple pharmaceutical companies as clients (Merck, Sanofi, GSK). A wide variety of samples have been used (gram negative and positive bacteria, virus, vaginal swabs, buccal cells, buffy coat blood, formalin fixed clinical tissues tissue, whole organisms, cells and fresh/frozen tissues). Validated assays of gene expression, DNA, and protein on the same array platform. PBA mixing for improved kinetics is being optimized. This protocol can be performed in 6 hr, sample to result. The probe/target hybridization step is performed in less than 15 min (versus 6 hr) the 384-well screening protocol and directly labeled probes work in a microRNA product that has just been launched, reducing the one step, and eliminating two steps that were part of the	Design of experiment (DOE) protocol initiated for on-chip mixing implementation. Demonstrated consistency of Agilent microarrays and qNPA assay on melanoma gene-set model using clinical samples (CV <10%), Assay has been multiplexed to measure 100 genes/well.

		6 hr TAT.	
Diagnostic Assay Format	4	Use of target specific detection probes and short incubations validated on the core diagnostic format, but not combined into a protocol, will provide a TAT of 4 hr. Microfluidic configuration will enhance sensitivity for possible detection in <4 h. and to achieve an LOD of 600 molecules. RNA, DNA and protein targets detectable in single assay;	Assay will be multiplexed for 100 pathogens, 5 genes/pathogen for low false positive rate. Testing at TRL 2
Upper Respiratory Pathogens Validated	2	Have validated use of buccal swabs, but have not handled other upper respiratory samples. Do not anticipate any issues.	Other infectious pathogen assays developed (HPV,….) Expecting signature data from EOS program, ANBC has contingency solution using high-density array
Sample Preparation Instrument	4	PBA sample prep module with self-contained fluidic actuation designed and tested. Benchtop development platforms validated for human cells. Infectious samples validated include gram negative and positive bacteria, HPV virus samples, vaginal swabs in Preservcite, formalin fixed tissues, buffy coat blood, red-cell lysed human blood, animal whole blood, buccal swabs, urine.	Prototype delivery to FBI labs on schedule for 7/1/07. Contract # FBI J-FBI-03-085-04/06 Mobile device for Forensic Science Services (FSS) under development.
Blood Sample Preparation	4	Direct detection from blood is possible (demonstrated for mouse blood), and is currently being developed for human blood.	Whole blood without separation (if needed) is TRL2
Upper Respiratory Sample Preparation	3	Buccal cells were used for qNPA. qNPA works well with fixed cells. Have validated the use of buccal swabs.	Experimental work with nasal washes and nasal swabs has not been performed yet (TRL 1).
Complete System Integration	2	System integration for other projects (FBI / FSS prototype for human ID) demonstrated. PBA integration in progress.	TRL3 expected in 6 months. Integration/testing to be demonstrated, alternative solutions identified.

OSMETECH MOLECULAR DIAGNOSTICS

Osmetech uses electrochemical technology for the detection of nucleic acids, eliminating the need for optical detection. The Osmetech proposal describes the OmniDx platform technology as highly multiplexed, enabling comprehensive test panels to be run on each sample including all necessary controls. The OmniDx will consist of two instrument modules– a sample prep module and a detection module, accompanied by three consumables, a very simple graphical user interface, and data analysis software. The OmniDx will be designed such that the consumables fit within the modules only in the correct orientation, and the software will lead the user through the minimal user interaction required to operate the system. The system will be designed to fit within 12" by 18" by 6", and will collectively weigh less than 25 pounds.

The Osmetech electrochemical technology is FDA cleared and future devices will be developed under the FDA's Quality System Regulation (QSR) utilizing existing sample preparation technology to develop the OmniDx, a small, fast, easy to use, highly portable, robust pathogen detection system with high specificity and sensitivity. The OmniDx will allow for automated sample preparation and the parallel detection of as many as 50-100 targets per sample.

The sample prep module will perform lysis of the pathogens, extraction and concentration of the nucleic acids, and perform reverse transcription to create cDNA. The first system to be developed will focus on swabs to ensure the presence of sufficient target pathogen for analysis and optimization. This will also allow for smaller sample volumes to be used in the first generation product. The sample prep module will account for the incorporation or loading of the sample, lysis of any pathogens present in the sample, extraction and concentration of the nucleic acid, and if necessary conversion of the RNA to DNA through reverse transcription. The output of the sample prep module will be DNA in solution that is ready for amplification and detection. The transfer of the DNA from the sample prep module to the detection module will be done manually with the use of a simple transfer device.

Pathogen lysis will take advantage of a variety of currently available methods, either through chemical or mechanical means. Chemical lysis can be quite effective, but it is usually necessary to modify the chemical mixture depending on the types of pathogens expected or anticipated for nucleic acid extraction from a sample. Mechanical methods can be more general, including the application of directed ultrasonic energy. Osmetech will explore both methods of lysis.

Magnetic beads will be utilized as the capture material for extraction in the sample prep station because it is possible to capture the nucleic acid from a large sample volume, pull the beads together into a small volume with a magnet, and elute the nucleic acid into a small volume of sample, thus effectively concentrating the extracted nucleic acids. The smaller, more concentrated sample enables a smaller detection unit with faster amplification and enhanced sensitivity. Once the nucleic acid is eluted from the beads, a reverse transcription step will be performed to convert any RNA in the sample to cDNA. Any and all reagents necessary for the sample prep step will be contained within a consumable in a stable form and will not need to be added or mixed by the end user.
The detection module will be based on established electrochemistry technology that allows for a high degree of multiplexing capabilities, which enables the end user to

screen or detect multiple pathogens simultaneously for each sample. This multiplexing capability also allows for the inclusion of positive and negative controls for each sample, which greatly increases the accuracy of and confidence in the results through quality control and assurance. Target nucleic acids will be hybridized to an array of electrodes modified with unique capture probes so that a single stranded target will be available after the amplification step. In this amplification strategy, the forward and reverse primers are not at equimolar concentrations in the PCR reaction. As the exponential amplification proceeds, one primer is exhausted first and the other primer then starts producing single stranded amplicons in a linear fashion. This single stranded species can then be hybridized to a capture probe at the electrode at a region interior to the primer sites. This method retains excellent specificity due to the three distinctive hybridization steps and allows for detection without any post-PCR treatment of the solution making this method suitable for a totally enclosed chip based assay.

It is possible to achieve a much higher degree of multiplexing by designing single stranded oligo probes that are present in solution during the PCR and have the ability to hybridize to capture oligos on an electrode as target amplification occurs. The location of the bound probe on the surface is indicative of the presence of a target specific sequence of nucleic acid associated with a particular pathogen of interest in the sample. The cleaved probe bound to the surface can then be detected using a variety of methods. A very simple, robust detection method is based on electrochemistry, where the capture nucleic acid is attached to an electrode and any cleaved probe that binds to the complementary nucleic acid on the surface is detected by measuring a current. The system to be developed under this proposal is based on this electrochemical detection method.

The electrochemical detection technology, which is incorporated into Osmetech's eSensor® Cystic Fibrosis Carrier Detection System, has been approved by the FDA for use in the clinical environment to detect carrier status of cystic fibrosis of adult couples contemplating pregnancy. This FDA-approved product demonstrates that this technology is acceptable to the FDA for clinical use and should ease the approval of future electrochemistry-based products. The Osmetech OmniDx technology platform is described in detail in the Osmetech White Paper, Development of a Universally Deployable Molecular Diagnostic System (OmniDx), found in Appendix O.

SCIENTIFIC ADVISORY BOARD TECHNOLOGY RECOMMENDATIONS FROM JULY 31, 2007 SAB MEETING

1.) In brief, because Akonni Biosystems failed to meet the EOS ACTD entry level requirement to test a mixture of one DNA virus, one RNA virus, one gram positive bacterium and one gram negative bacterium, the SAB recommends no further funding or engagement with Akonni towards fulfillment of EOS ACTD objectives at this time. Maj Dempsey reminded the MST that Akonni is still on contract and may perform one additional test to meet this requirement.

2.) The SAB recommends review of the Nanogen White Paper and no further action until a decision is made whether to request a full proposal under the Fall BAA.

3.) Cepheid is working toward an FDA cleared pandemic surveillance and diagnostics influenza panel capability on the GeneXpert system. The SAB recommends going forward with determining the specific requirements for a no cost proposal to collaborate with Cepheid to develop the FluXpert cartridge.

4.) Assuming receipt of a BAA proposal in Fall 07, the SAB recommends going forward with ITI with the FilmArray to meet upper respiratory multi target/agent testing objectives with integrated extraction by May, 2008 and overlapping BT agent testing, all working toward FDA approval. One option is to extend the ACTD a year to meet ACTD level 3 exit criteria with the FilmArray.

5.) Osmetech requested funding over 3.5 years to develop a multi target/agent analysis with an independent sample processing device, and will seek FDA approval once the technology is developed, but cannot provide a system to meet ACTD requirements under the current timeline or with a one year extension of the criteria. SAB members recommend going forward with Osmetech under the current BAA proposal under a highly structured deliverable/milestone basis.

The SAB did not provide recommendations on the ASU PBA because this technology did not meet entrance criteria for the ACTD, nor was a future technology of interest for EOS AF/SGR. Meso Scale Diagnostics was not evaluated by the SAB because they did not submit a white paper for review or a broad area announcement (BAA) proposal for funding. MagnaPure Biosciences requested to be taken out of the review process until their technology matures further.

Other SAB summary comments:

1.) Dr. Estacio noted the need to manage informatics and a plan for collaborations, search engines on EOS data. For the future success of IT knowledge management, he suggested a modular approach to allow different platform outputs to input into COHORT via an appropriate platform specific adaptor. A good modular approach will allow the next generation platform to build to well-defined requirements with the lowest cost burden. He also commented that secure collaboration portals can accelerate work

progress with built in version controls and automatic team alerting with updates in program elements.

2.) Gen Casey noted that the USAF has knowledge sharing systems that need to be addressed in the Strategic Plan as well.

3.) Liz Spangler reminded the group of the need to upgrade the ADL link and that the bandwidth limitation needs to be resolved.

4.) It was noted that COHORT is taking data every 5 min, while other data systems are satisfied with taking data every 12-36 hours. This is a significant difference in the definition of real time systems.

AF/SGR Strategic Plan- The Future Vision and Roadmap for Biological Threat Surveillance and personalized Diagnostics for AF/SGR FY08-FY20

The objective of the Strategic Plan is to provide a strategic plan to research, develop, implement, and transition a system of systems for advanced biological threat surveillance and personalized diagnostics for AF/SGR with a deliverable date of 31 December, 2007.

The Strategic Plan will be organized into 14 subject area TABS as follows:

Tab 1&2:	Executive Summary & Strategic Plan
Tab 3:	Capability Gaps and Requirements Overview
Tab 4:	Advanced Molecular Diagnostics
Tab 5:	IT
Tab 6:	Surveillance
Tab 7:	Decision Support Tools
Tab 8:	FDA Regulatory Path
Tab 9:	Market Entry/Commercialization
Tab 10:	Implementation
Tab 11:	Science Advisory Board
Tab 12:	Transition
Tab 13:	ELSI
Tab 14:	Projected FIN and Timeline (BAA)

Lackland AFB/WHMC
Epidemic Outbreak Surveillance (EOS)
2460 Pepperell St., Bldg. 4429
Lackland AFB, TX 78236

EOS Operating Instruction 001
27 January 2004

Collection of Nasal Wash and Throat Swabs in Basic Military Trainees and Hospital and Clinic Patients

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This instruction provides the staff of Epidemic Outbreak Surveillance (EOS) specific guidance for enrolling patients, collecting specimens, and logging specimens for the IRB approved research study.

1. References: Wilford Hall Medical Center (WHMC) Protocol For Clinical Investigation--Human: Rapid Diagnosis of Respiratory Viruses in Hospital and Clinic Patients, 59th MDW Medical Center Instruction (MCI) 44-9, Infection Control Guidelines.

2. Setting: Ward 9D, Infectious Diseases Clinic, Internal Medicine Clinic, WHMC Family Practice Clinic, Emergency Department, and Fast Track.

3. General: Standard precautions will be used when collecting specimens from patients. EOS staff will wear at a minimum, a standard hospital facemask and gloves when performing a nasal wash.

4. Consenting Patient:
EOS Personnel Required: Advising Investigator

4.1. Discuss purpose of the research study as indicated on the Informed Consent Document. *(Attachment 1).*
4.2. Give the patient a copy of the Informed Consent Document to review.
4.3. Be prepared to answer questions pertaining to study.
4.4. If the patient agrees to participate in the study the patient will provide his/her signature, printed name, social security number, family member prefix (FMP), sponsors social security number, date of enrollment, and date of birth on page 4 of the informed consent document.
4.5. The advising investigator must also provide his/her signature, printed name, date of patient enrollment, and work number on page 4 of the informed consent.
4.6. A witness must provide signature, printed name, and date of patient enrollment on page 5 of the informed consent. The witness may not be anyone associated with the research study. The witness can be a family member or other hospital staff.
4.7. Discuss the reason for the Authorization To Use and Disclose Protected Health Information form with the patient. *(Attachment 2).*
4.8. The patient will provide signature, printed name, volunteer's social security number, date authorization given, and sponsor's social security number on page 3.

4.9. A witness must provide signature, printed name and the date of authorization given on page 3 of the form. The witness may not be anyone associated with the research study. The witness can be a family member or other hospital staff.

5. Ordering Flu Nasal Wash in CHCS

Note: The ordering provider or nursing staff must order flu nasal wash test in CHCS.

5.1. At lab test prompt enter "flu"
5.2. Select # 3, Flu A/B EIA Panel
5.3. Select # 4, Nasal washing cup

6. Nasal Wash and Throat Culture Procedure *(attachment 3)*:

6.1 Nasal Wash Protocol

6.1.1. Explain procedure to patient
6.1.2. Draw 5 cc of normal saline into a 10 cc syringe.
6.1.3. Put a blue drape on the patient around the neck/chest.
6.1.4. Give the patient a sterile container to hold in their right hand (lid off).
6.1.5. Have the patient tilt their head back, press the tongue up against the soft palate and take a breath and hold it.
6.1.6. Insert the tip of the syringe into one nostril and instill the 5 cc of normal saline over approximately 3-5 seconds (instill the normal saline solution at a steady pace so that it is not too fast, but quickly enough so the patient will not have to hold their breath for an extended amount of time).
6.1.7. Then have the patient lean forward, with the sterile cup positioned under the nostrils and allow the normal saline to drain back out of the nasopharynx into the sterile cup. You should get about 1½ cc of solution back. The reason for instilling 5 cc or so is to get the solution to go back into the nasopharyngeal area (you want to wash the pharynx too). You definitely will not get all of the solution back-some of it will be absorbed and some will be swallowed.
61.8. Label specimen with patient's name, family prefix, SSAN, and location where specimen was collected

6.2. Throat Swab Protocol

6.2.1. Explain procedure to patient
6.2.2. Use the viral culture swab (green tip).
6.2.3. Rub swab firmly on both tonsils and soft palate/pharynx.
6.2.4. Place swab in container and press tip of container to release transport media.
6.2.5. Label specimen with patient's name, family prefix, SSAN, and location where specimen was collected

6.2.6. Hand carry viral swap to lab central operations.
Note: It is important to press firmly with the swab and to sample the entire pharyngeal area.

7. Logging in Specimens at Central Operations Main Lab

7.1. Log specimens in the specimen log book at lab central operations.
7.2. Have lab technician enter information into CHCS and print labels.
7.3. Take the nasal wash and viral culturette to the microbiology lab.
7.4. Attach small lab label to the viral culturette and to the top of nasal wash container.
7.5. Attach large lab label to the side of nasal wash container.
7.6. Give nasal wash to the lab technician who is performing the influenza A-B test.
7.7. Place the viral culture in the EOS holding basket in the refrigerator.

8. Preparing Daily Specimen Collection Sheet:

Items Needed: Microbiology Lab Flu Book
EOS Hospital Influenza Study Collection/Processing Sheet *(Attachment 4)*

8.1. Find matching pairs of nasal washes and viral cultures located in EOS basket in microbiology refrigerator
8.2. Find all positive nasal washes without viral cultures.
8.3. Discard all specimens that are from patients 17 years old or younger.
8.4. Discard all non-positive nasal washes.
8.5. Discard all specimens that are over 36 hours old.
8.6. Log matching pairs (nasal washes and cultures) on EOS log sheet first.
8.7. Log acceptable positive nasal washes next.
8.8. Annotate your name, date and time on top of EOS sheet.
8.9. Fax sheet to EOS Bld. 1245, @ 210-671-0649.
8.10. Review lab flu book for basic training patients that might have been tested for influenza A-B.
8.11. Contact Protocol Coordinator if there are any trainee’s with positive nasal washes.

9. Transporting Specimens to Clinical Investigation Directorate (CID)

9.1. Place specimens in acceptable bio-hazard bag
9.2. Hand carry specimens to CID laboratory Bld. 4430 Rm. 197 and place in refrigerator (put on gloves prior to entering room)

Attachments:

1. Wilford Hall Medical Center Informed Consent Documents
   (ICD Template Version 4. Feb 02)
2. Authorization To Use and Disclose Protected Health Information
3. Nasal Wash and Throat Culture Protocol Instruction Sheet
4. EOS Hospital Influenza Study Collection/Processing Sheet
5. Recommendations for Ordering Nasal Washes for Influenza

Paul Kittle, TSgt. USAF
NCOIC, Epidemic Outbreak Surveillance

Date Signed: _______________

Approved/Disapproved

Reviewed: _______________

Reviewed: _______________

7-February-2007

DEPARTMENT OF THE AIR FORCE
Epidemic Outbreak Surveillance (59 MDW/CM)
Advanced Diagnostic Laboratory
2460 Pepperell Street, Bldg. 4429
LACKLAND AIR FORCE BASE, TEXAS 78236-5300

MEDICAL

EOS Clinical Sample Preparation "Aliquotting"

ADL Operating Instruction 44COL-02 (Revised)

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1. **Purpose**:
This operating instruction (OI) establishes a standard method for handling and aliquoting respiratory pathogen samples collected by the clinical staff for use in Nucleic acid extraction, shipping and storage of samples for an extended time period.

2. **Specimen**:
3 ml of Nasal Wash (WS) collected on patients and 1.5 ml of normal saline solution containing Throat Swab (TS). Samples are stored at 4º Celsius until aliquotted.

3. **Supplies and Equipment**:

3.1. Biosafety cabinet level 2 or greater
3.2. Wheaton sterile 2 ml cryovials with caps
3.3 Remel MicroTest™ M4RT® Multi-Microbe Media Transport Tubes
3.4. Eppendorf repeater pipette
3.5. Eppendorf combitips, 5 ml
3.6. Wheaton cryovial racks
3.7. Biohazard waste bags
3.8. Gloves and laboratory coat
3.9. Sleeve protectors
3.10. 4" * 4" zip-lock bags
3.11. Biohazard shipping bags
3.12. Inventory log sheet
3.13. Pre-printed labels
3.14. Ethanol Solution, 70%
3.15. Decon® Disinfectant
3.16. Tweezers
3.17. Sani-cloth Plus wipes (Bactericidal, Tuberculocidal, and Virucidal)

OPR: 59MDS/CM (Mr. Fugate) Certified By: 59MDW/CM (Lt Col Livingstone)
Pages: 5/Distribution: All ADL Personnel

4. Quality Control

Samples are handled in a bio-safety cabinet using sterile techniques. Combitip pipette tips are changed between samples. Splashing of samples is eliminated by controlling the dispensing force of sample from the pipette into the cryovial. Cross contamination of samples is prevented by using bio-storage bags and by avoiding touching the combitips to other vials.

5. Setting up Procedure:

Note: Don gloves prior to entering the BSL-2 room.

5.1 Gather and place in bio-safety cabinet, blue under pad, biohazard bag, sani-wipes, 70% ethanol cup, Saline tube and tweezers.

5.2 Set up cryovial tubes (Yellow cap) for NW and (Red cap) for TS in a cryovial rack.

5.3 Place disinfecting solution bottle next to hood along with wipe towels and tape.

5.4 Put on personal protective equipment in order: Gown/Lab coat, second pair of gloves and sleeve protectors.

5.5 Remove samples from the refrigerator and place them (it) in the Bio-Safety Cabinet.

5.6 Confirm the sample being processed with the PIN number label that was printed prior to aliquotting. To confirm the correct sample being processed belongs to the correct label.

Note:

- Clean gloves with 70% Ethanol if they come into contact with any sample.
- Clean tweezers with 70% Ethanol after each sample when using them to extract the throat swab from the conical tube.
- Clean gloves with 70% Ethanol after all aliquotting is completed prior to any other clean up.

6. Aliquoting Procedures:

6.1 Aliquoting Nasal Wash Samples for Storage and Shipment:

6.1.1 Set up (9) sterile yellow cap cryovials and one MicroTest™ M4RT® Transport Media tube in the rack for each NW specimen received.

6.1.2. Place the corresponding “A” label on the first cryovial and “B” label on Transport Media tube.

6.1.3. Add combitip to the Eppendorf Repeater Pipette and set dial on pipette to dispense 100µl.

6.1.4. Draw up NW sample into pipette (up to 5ml volume).

6.1.5. Dispense 200µl of nasal wash sample into the A cryovials and B on Transport Media tube by depressing the thumb lever twice into each vial. Note: (100µl is dispensed with each depression of the thumb lever on the pipette).

6.1.6. For samples 1 thru 7 dispense 100µl into each cryovial, for cryovial 8 dispense the remaining amount up to 1.8 ml. **NOTE**: ((For small volume samples if there is not enough NW sample to fill all the cryovials, aliquot 200µl into the first cryovial and Transport Media tube. Then add 100µl to as many of the remaining vials as possible. Note: (Annotate volume differences for the last cryovial in the special vol. block for that specimen).))

6.1.7. Any remaining NW sample will be added to Transport Media tube for shipment to reference facility for culture.

6.1.9. Discard pipette into biohazard waste bag.

6.2 Aliquoting Throat Swab Samples:

6.2.1. Set up (7) sterile Red cap cryovials and one MicroTest™ M4RT® Transport Media tube in the rack for each throat swab specimen received. Place the corresponding “A” label on the first cryovial.

6.2.2. Open the 15ml conical centrifuge tube and add additional saline bringing the volume up to 2.5ml.

6.2.3. Using the tweezers pull the swab up about half way, then using a sani-wipe press the swab against the side of the tube to squeeze as much liquid out of the swab as possible. Remove the swab and discard it in the biohazard waste bag.

6.2.4. Then while tilting the conical tube pour the solution into the 6th cryovial tube.

6.2.5. Add combitip to the Eppendorf Repeater Pipette and draw up the solution.

6.2.6. Dispense 200µl of throat swab sample into the A cryovial and B Transport Media tube by depressing the thumb lever twice into each vial. **Note**: (100µl is dispensed with each depression of the thumb lever on the pipette).

6.2.7. Dispense 100µl of the remaining solution into cryovials 1 thru 5 and in cryovial 6 dispense the remaining solution up to 1.8 ml. **Note**: (Annotate volume differences for the last cryovial in the special vol. block for that specimen).

6.2.8. Discard pipette into biohazard waste bag.

6.2.9. Replace all cryovial caps and using the 70% Ethanol spray bottle spray down the cryovials and rack(s). Then using the sani-wipes wipe down the rack(s) and the cryovials.

6.2.10. Remove rack(s) from the BSL-2 Biosafety Cabinet and place them on the counter.

7. Clean up Procedures for the P2/BSL-2 Hood:

7.1 Using the 70% Ethanol spray bottle spray off the Eppendorf Repeater and wipe it off with one of the sani-wipes, then place it back into the supply box.

7.2 Place all disposable/contaminated supplies into the biohazard bag.

7.3 Use 70% Ethanol to decontaminate the inside of the hood and surfaces. Wipe off excess 70% Ethanol with a white paper towel and dispose in the biohazard bag.

7.4 Twist the top of the biohazard bag and seal the top with tape to secure.

7.5 Remove biohazard bag from biosafety cabinet and place it in the large biohazard trash receptacle.

7.6 Turn-on UV lights with interior gloves, remove and discard prior to leaving the BSL-2 Room.

7.7 Upon completion of aliquoting process secure the BSL-2 area to prevent unauthorized entry.

Attachment 1: LABELING, STORAGE & DATA ENTRY

Edited By:

W. Howard Fugate, B.S., MBA
ADL, Laboratory Manager

Approved By:

Lisa Lott, PhD
Principal Investigator

Reviewed By:____Date:__________________

Reviewed By:____Date:__________________

Reviewed By:____Date:__________________

Reviewed By:____Date:__________________

Reviewed By:____Date:__________________

SGUR 0.1 Layout

ABI TaqMan Low Density Respiratory Pathogen LDA
SGUR 0.1

		Neg Cont	Samp 1	Samp 2	Samp 3	Samp 4	Samp 5	Samp 6	Samp Pos C
Flu A (USAF)	**1**	T B	T B	T B	T B	T B	T B	T B	T
Flu A - H1 (USAF)	**2**	T B	T B	T B	T B	T B	T B	T B	T
Flu A - H3 (USAF)	**3**	T B	T B	T B	T B	T B	T B	T B	T
Flu A - H5.1 (USAF)	**4**	T B	T B	T B	T B	T B	T B	T B	T
Flu A - H5.2 (USAF)	**5**	T B	T B	T B	T B	T B	T B	T B	T
Flu B (USAF)	**6**	T B	T B	T B	T B	T B	T B	T B	T
SARS.1 (CDC)	**7**	T B	T B	T B	T B	T B	T B	T B	T
SARS.2 (CDC)	**8**	T B	T B	T B	T B	T B	T B	T B	T
RSV A (Eagle)	**9**	T B	T B	T B	T B	T B	T B	T B	T
RSV B (Eagle)	**10**	T B	T B	T B	T B	T B	T B	T B	T
Sample Control (RNP) (CDC)	**11**	T B	T B	T B	T B	T B	T B	T B	T
Adeno - pan (CDC)	**12**	T B	T B	T B	T B	T B	T B	T B	T
Adeno - 4 (Eagle)	**13**	T B	T B	T B	T B	T B	T B	T B	T
HPIV (Eagle)	**14**	T B	T B	T B	T B	T B	T B	T B	T
Buffer Blank	**15**	T B	T B	T B	T B	T B	T B	T B	T
HMPV (Eagle)	**16**	T B	T B	T B	T B	T B	T B	T B	T
Strep pneumo (CDC)	**17**	T B	T B	T B	T B	T B	T B	T B	T
Strep pyogenes (CDC)	**18**	T B	T B	T B	T B	T B	T B	T B	T
Mycoplasma pneumo (CDC)	**19**	T B	T B	T B	T B	T B	T B	T B	T
Chlamydia pneumo (CDC)	**20**	T B	T B	T B	T B	T B	T B	T B	T
Bordetella pertussis I (CDC)	**21**	T B	T B	T B	T B	T B	T B	T B	T
Bordetella pertussis II (CDC)	**22**	T B	T B	T B	T B	T B	T B	T B	T
Legionella pneumo (CDC)	**23**	T B	T B	T B	T B	T B	T B	T B	T
Armored RNA (XenoRNA)	**24**	T B	T B	T B	T B	T B	T B	T B	T

(yellow)	**Respiratory Pathogen Primer/Probes**	(grey)	**Clinical Sample Control**
(pink)	**Positive Sample Control**	(black)	**Clinical Sample Extraction Control (Armored RNA)**
(cyan)	**Negative Sample Control**	**B**	**BHQ Quencher**
CDC Assays		**T**	**TAMRA Quencher**
Eagle Assays			

CLINICAL LABORATORY

Virus Inoculation: Influenza Surveillance Program

(formerly called "Project Gargle")

1. PRINCIPLE:

1.1. The Air Force Surgeon General-directed respiratory surveillance program, originally referred to as "Project Gargle," monitors the influenza strains circulating in the population at any given time. The program is administered by the Disease Surveillance Branch (RSRH). Only the laboratory procedures are dealt with here.

1.2. This is now a tri service program that is funded by the Global Emerging and Infections System (GEIS) with the Air Force as the lead agent.

1.3. The primary objective of the program is to monitor influenza, but detecting any respiratory virus provides clinical as well as epidemiological information for clinicians and RSRH.

2. SPECIMENS:

2.1. Throat swabs or nasal washings (Attch 1) should be collected within the first 48-72 hours of onset from patients presenting with the following symptoms:

2.1.1. Fever of >/= 100.5° F oral or equivalent
2.1.2. Cough or sore throat
2.1.3. Any individual with acute pneumonia or symptoms compatible with influenza activity.

2.2. Samples that will reach the laboratory within 4 days of collection may be stored at refrigerated temperatures (4^0 C) and shipped on gel packs. If the collection to laboratory receipt time will be >4 days the samples should be stored at -70^0 C and shipped on dry ice.

2.3. In-house study

3. REAGENTS, SUPPLIES, AND EQUIPMENT:

Supersedes:28 January 2004
Number of Pages: 11 and 2 attachments
OPR: AFIOH/SDEM
Approved By: Elizabeth A. Macias, Ph.D., D(ABMM)
Author: Linda C. Canas
Distribution: Virology Procedure Manual

Summary of changes: Renumbered, formerly 44-5006. Reporting results procedure has changed.

3.1. MicroTest M4™ transport medium. This is supplied to customers on request.

3.2. Incubator, 34^0 - 37^0 C

3.3. Swinging bucket centrifuge

3.4. Laboratory refrigerator

3.5. Fluorescent microscope with filters for FITC

3.6. Humid chamber

3.7. Sterile Pasteur pipettes

3.8. Sterile transfer pipettes

3.9. Sterile graduated pipettes; 10 ml, 5 ml, 1ml, combitip

3.10. Acetone, reagent grade

3.11. Specimen processing medium: Eagles' Minimal Essential Medium (MEM)

3.12. Penicillin/Streptomycin

3.13. Gentamycin

3.14. Fungizone

3.15. Fetal Bovine Serum (FBS)

3.16. 12 X 75 sterile polystyrene capped tubes

3.17. Colored dots to fit tube caps

3.18. Sterile cryovials

3.19. Primary tissue culture (primary Rhesus Monkey Kidney-PMK or Cynomologous Monkey Kidney-CYN are most commonly used).

3.20. Madin Darby Canine Kidney (MDCK) shell vials (SV)

3.21. Vero cells

3.22. A549 tissue culture tubes

3.23. Shell vials with primary tissue culture

3.24. Sterile phosphate buffered saline (PBS)

3.25. Multi-well Teflon coated slides

3.26. No. 1.5 coverslips

3.27. Anti-viral antibody screening reagent containing mouse monoclonal antibodies directed against adenovirus, influenza A, influenza B, parainfluenza types 1,2,3, and RSV.

3.28. FITC labeled anti-mouse antibodies with Evan’s blue counterstain for each respiratory virus in 3.27 above.

3.29. Non-immune mouse antibodies.

4. CALIBRATION: N/A

5. QUALITY CONTROL:

5.1. Shell Vials

5.1.1. One shell vial is inoculated with either influenza A, B, parainfluenza 1,2, or 3 in a daily rotation following the schedule outlined below.

5.1.2. At least one shell vial per day is inoculated with the negative control prepared from an uninoculated M4 collection tube. (OI 44-5004)

5.2. Staining reagents

5.2.1. When a new lot number or new shipment of reagents is received, the reagent must be QC'd and labeled as OK before being placed in the rack for use.

5.2.1.1. Stain a purchased slide covering the seven viruses in the respiratory screen stain (flu A,B, para 1,2,3, adeno, RSV) and a negative well.

5.2.1.2. Each monoclonal stain is tested using a purchased slide that covers all seven viruses listed above.

5.2.2. Positive and negative controls must be run on each day of fluorescent antibody (FA) staining.

5.2.2.1. Respiratory screen positive controls must be run every day the test is performed.

5.2.3. When specimens are set up in shell vials, one virus is tested each day in a rotation with both the screening stain and the corresponding monoclonal stain so each reagent is thoroughly tested in a given week.

SET UP	INOCULATE	STAIN
MONDAY	Parainfluenza 1	Wednesday
TUESDAY	Parainfluenza 2	Thursday
WEDNESDAY	Parainfluenza 3	Friday
THURSDAY	Influenza B	Monday
FRIDAY	Influenza A	Monday

5.2.4. Adenovirus and RSV are not covered by this plan. A control slide must be stained each day of patient testing.

5.3. Degree of fluorescence and staining patterns must be typical of the virus tested. Charts with that information are posted by the microscope and are addressed in OI-44-5012.

5.4. Negative controls must show a red background with an absence of fluorescence

5.5. Each day, inoculate 1 tube of Vero cells and one SV of Madin Darby Canine Kidney (MDCK) cells with the positive and negative respiratory controls for that day. These cells are not routinely used in this laboratory, but may be needed for SARS (Vero) or influenza (MDCK) if the PMK cells are not available. These tubes are included so we are familiar with the cells in case we have to bring them in-house for routine use.

5.6. The virus used in the shell vial for a positive control is also inoculated into the PMK tissue culture tube. It is used as a reference for CPE and as a positive control for the hemadsorption procedure.

6. **PROCEDURE:**

6.1. Processing specimens

6.1.1. Upon receipt in the laboratory, all specimens are checked to assure the label generated in shipping and receiving has the same information as the original patient label.

6.1.2. Using the computer generated labels, label the worksheet and 12 X 75 processing tube.

6.1.3. Label the colored dot on the 12 X 75 tube with the computer number.

6.1.3.1. Label a cryo-vial with a HIPAA number and "dot" it with the patient accession number that is used on the 12 X 75 tube. This "dot" will be removed when all testing is complete. At that point, the HIPAA number will be used in all subsequent references.

6.1.3.2. Add ~0.5ml of the original specimen to the HIPAA labeled cryo-vial.

6.1.3.3. Freeze this cryo-vial at -70°C and save for possible further testing.

6.1.3.4. Add the 2ml of processing medium (MEM + antibiotics) that is in the 12 X 75 labeled tube to the remaining specimen received in transport medium; vortex 15-30 seconds.

6.1.4. Allow processed specimen to sit at 4°C for a minimum of 30 min before inoculating tissue culture.

6.2. Procedure when shell vials are used.

6.2.1. Aspirate feeding medium from labeled shell vial and tissue culture tubes.

6.2.1.1. Inoculate one PMK shell vial with 0.5 ml processed specimen and 1 ml feeding medium that has no added FBS.

6.2.1.2. Arrange vials to balance in a swinging bucket centrifuge and centrifuge 60 minutes at 700 rpm in the R&B Silencer® 2200R centrifuge

6.2.1.3. Arrange vials in racks and incubate at 35°C 2- 4 days (NOTE:2 days is the norm, but weekends and holidays can be accommodated)

6.2.2. Decant feeding medium from one tube of PMK and one tube of A549 tissue clulture.

6.2.2.1. Add 2 ml fresh feeding medium + antibiotics to each tube of tissue cuture.

6.2.2.2. Inoculate each labeled tube of tissue culture with 0.5 ml of the corresponding patient specimen

6.3. Staining shell vials.

6.3.1. Aspirate MEM and replace with 1 ml PBS from dispenser bottle

6.3.2. Repeat. Aspirate and add 1 ml PBS.

6.3.3. Let set 10 minutes at RT.

6.3.4. Aspirate the second PBS rinse and replace with 1 ml fresh PBS. Leave this third addition of PBS in the shell vial.

6.3.5. Using a sterile Pasteur pipette, gently scrape the cover-slip to dislodge the cells.

6.3.6. Label the slide map (Attachment 2)

6.3.7. Label 12 well slides with slide # to match map

6.3.8. Record lot numbers and expiration dates of all stains used

6.3.9. After double checking specimen SV ID, slide location, and slide map location, spot one drop of cell mixture onto well. Spot two wells with the known positive virus.

6.3.10. Recap vials and store at 4°C.

6.3.11. Allow slides to air dry in the biosafety hood away from the grate.

6.3.12. Fix slides with reagent grade acetone; place on slide warmer until excess acetone has evaporated.

6.3.13. Referring to the slide map, apply 1 drop of the control or respiratory screen fluorescent antibody stain to the corresponding labeled slide well.

6.3.14. Incubate 30 minutes at 35 - 37°C in a humid chamber.

6.3.15. Rinse in PBS 5 min and then briefly in sterile water. Tap to remove excess water. Dry on slide warmer.

6.3.16. Using a drop of mounting fluid on each well, cover-slip the wells and examine the slides using the FITC filter on the fluorescent microscope. Record results on slide map.

6.4. Interpretation

6.4.1. Spot two wells with the known positive virus. Stain one with the respiratory screen stain and one with the monoclonal stain for the target virus. Both should stain substantially the same.

6.4.2. Positive wells are determined by degree of fluorescence as well as a pattern typical of the virus. Fluorescence is graded on a 1+ - 4+ scale and this refers to the intensity of fluorescence. Staining intensity should be at least a 2+ to be called positive.

6.4.3. Negative wells will have red cells and no evidence of fluorescence.

6.5. Record the results of the respiratory screening stain on the worksheet for each sample.

6.5.1. FA screen Positive from the SV:

6.5.1.1. Go back to stored SV and prepare a panel slide; stain with monoclonal reagents. If there is a predominant isolate for a given location (for example Flu A) the positive pool specimen can be stained for that virus only. If that result is negative, the entire panel slide is then prepared.

6.5.1.2. If shell vial is positive at 48-72 hours, the worksheet is marked with the isolated organism and a final report is entered into the computer.

6.5.1.3. If the SV is positive, but the tissue culture tubes show no sign of viral infection, mark the worksheet with the SV results, report results and continue to follow the tubes. An isolate is needed for archiving.

6.5.1.4. The SV from influenza A positive specimens should be given to the influenza molecular team for further characterization.

6.5.2. If shell vial is negative, note this on the individual worksheets and continue to examine the tubes at a minimum on days 2, 5-7 and 9-10.

6.6. Tubes showing CPE that are not already identified in the SV can be further tested by several methods:

6.6.1. Hemadsorption (OI-44-5010)

6.6.2. Fluorescent antibody stain for virus consistent with the CPE exhibited (OI-44-5012)

6.6.3. Primary tubes with no evidence of CPE must be screened by a hemadsorption (HAd) test on day 9-10. (OI 44-5010).

6.7. Procedure when shell vials are not used.

6.7.1. Reasons shell vials would not be used:

6.7.1.1. During the summer months the incidence of respiratory disease is greatly reduced and the shell vial procedure is designed for large numbers of specimens.

6.7.1.2. The number of specimens exceeds the number of shell vials available.

6.7.2. Two tubes of primary tissue culture and one A549 tube are set up.

6.7.3. Tubes are examined for CPE on day 1, 2 and every other day until reported

6.7.4. A hemadsorption test is performed between day 2-7 on one of the primary tubes.

6.7.5. If there is no evidence of viral infection on day 10, prepare a slide and stain with the respiratory screening FA stain as a last screening step before reporting results.

7. CALCULATIONS: N/A

8. REPORTING RESULTS:

8.1. Results are entered into CHCS through **^ERA** (Lab Result Entry)
Select Accession: **VRV**
Result Type: **(F)inal** or **(I)ntermediate**

8.1.1. Virology Result: *Negative results are entered here.*
F9 key
NRV *codes for No Respiratory Virus Isolated*

8.1.2. If the accession is positive,
Enter
Select Virus: *Enter the name of the virus. If a pick list comes up use the arrow keys to move to the desired name.*
Select
DO
(Q)uit for the person entering results or
(C)ertify for the person reviewing results.

8.2. The results should be entered by one person and certified by another. If there are not two people, results should not be held up and the same person may enter and certify.

8.3. The Supervisor will review all results.

8.4 When subtyping results become available, the original report must be amended.

8.4.1.The accession date is required for this.
^RRA Review Results by Accession.
Select Accession: **VRV**
Accession Numbers: *enter the accession numbers for those reports to be amended. Record the accession date for each one.*

8.4.2. **^AMR** Amend Results
Select Accession Date**: month/day/yr**
Select Accession Area: **VRV**
Select Accession Number: **XXX**
Amend these results? Y//**Yes**
Review accession comments?// **Yes** or **No**

8.4.3. Page down to the Select Virus Field which will already have the influenza virus result.

8.4.4. Enter **Influenza.** This will bring up the result already entered
INFLUENZA VIRUS TYPE A
OK? Y
Enter **(N)o** and the dictionary of influenza viruses will appear.
Using the down arrow key, page down to the correct subtype
Select
Using the up arrow key, go to the original entry without the subtype.
Remove
OK to DELETE the entire INFLUENZA VIRUS TYPE A Entry?
Yes
Do
(C)ertify

9. PROCEDURE NOTES:

9.1. Influenza isolates from all overseas locations, representative samples from stateside locations, as well as early and out of season isolates, are selected for sub-typing.

9.1.1. Influenza A isolates may be subtyped by PCR (influenza molecular department) or Hemagglutination-inhibition (OI 44-5016).

9.1.2. Influenza B isolates are subtyped by Hemagglutination-inhibition.

9.2. Sometimes the positive control in the shell vial is no longer viable and the results of the FA staining will be negative. In such cases, use a prepared control slide to determine the acceptability of the stain and the results for patient shell vials for that day.

10. LIMITATIONS OF THE PROCEDURE:

10.1. If the specimen is taken too late in the illness, viable virus may no longer be shed and a viral diagnosis cannot be confirmed

10.2. Specimens that have been mishandled may result in nonviable virus reaching the laboratory. This is not always evident in the laboratory.

10.3. Not every respiratory virus is covered by this procedure. We can reasonably expect to isolate influenza, parainfluenza, adenovirus, enterovirus, and herpes simplex virus. RSV is occassionaly isolated, but it is expected to be detected by rapid tests at the local laboratory.

11. REFERENCES:

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11.2. Light Diagnostics Respiratory Panel 1 Viral Screening & Identification Kit. Diagnostic Kit Package Insert. Distributed by Chemicon International, Inc., 28835 Single Oak Drive, Temecula, CA 92590.

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11.4. Murray,Patrick, Baron,Ellen Jo, Jorgensen,James, Pfallelr,Michael, Yolken,Robert Editors. *Manual of Clinical Microbiology. 8th Edition.* 2003. ASM Press. 1752 N St. NW, Washington, DC 20036-2094. pp1360-1374.

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Streptex* ZL50/61

Rapid latex test for the qualitative detection and identification of the Lancefield group of Streptococci

STREPTEX*

INTENDED USE

Streptex* is a rapid latex test system for use in the qualitative detection and identification of the Lancefield group of streptococci. Reagents are provided for groups A, B, C, D, F and G covering the majority of clinical isolates[6]: group E streptococci are rarely isolated.

SUMMARY AND EXPLANATION OF THE TEST

The majority of species of Streptococcus possess group-specific antigens which are usually carbohydrate structural components of the cell wall. Lancefield showed that these antigens can be extracted in soluble form and identified by precipitation reactions with homologous antisera[12]. Several procedures for extracting the antigens have been described[3,4,10,13,17,18]. In the Streptex* system a simple enzyme extraction is employed and a more rapid acid extraction system is available (Streptex* Acid Extraction Kit, ZL59/9E13-01).

The main use of the test is in identification of streptococci growing on agar plates, but satisfactory results have been reported with one hour extraction from pure broth cultures[8].

PRINCIPLE OF THE PROCEDURE

Group specific antigens are extracted from streptococci in a simple incubation step. Antigens are then identified using polystyrene latex particles which have been coated with group-specific antibodies. These latex particles agglutinate strongly in the presence of homologous antigen, and remain in smooth suspension in the absence of homologous antigen.

KIT CONTENTS

Streptex*	50 tests (ZL50/9E05-01)	200 tests (ZL61/1F47-01)
1. Group A Latex (ZL51/9E06-01)	1 dropper bottle (light blue cap)	4 dropper bottles (light blue caps)
2. Group B Latex (ZL52/9E07-01)	1 dropper bottle (pink cap)	4 dropper bottles (pink caps)
3. Group C Latex (ZL53/9E08-01)	1 dropper bottle (brown cap)	4 dropper bottles (brown caps)
4. Group D Latex (ZL54/9E09-01)	1 dropper bottle (dark blue cap)	4 dropper bottles (dark blue caps)
5. Group F Latex (ZL56/9E11-01)	1 dropper bottle (grey cap)	4 dropper bottles (grey caps)
6. Group G Latex (ZL57/9E12-01)	1 dropper bottle (yellow cap)	4 dropper bottles (yellow caps)
7. Polyvalent Positive Control (ZL58/1F46-01)	1 dropper bottle (red cap)	2 dropper bottles (red caps)
8. Extraction Enzyme (ZL55/9E10-01)	2 bottles	8 bottles
9. Disposable Mixing Sticks	3 bundles	12 bundles
10. Disposable Reaction Cards (RT02/3F86-01)	2 packs	8 packs
11. Instructions for Use	1	1

REAGENTS

STORAGE AND LIFE

A removable storage rack holding all the reagents which require refrigeration is provided with each kit. A large storage rack is provided in the ZL61/1F47-01 kit for storing bulk reagents. Unless otherwise stated all reagents should be stored at 2 to 8°C, under which condition they will retain activity until the expiry date of the kit.

Latex Suspensions

Six (ZL50/9E05-01) or four sets of six (ZL61/1F47-01) plastic dropper bottles, one specific for each of the groups A, B, C, D, F and G, each containing minimum of 1.2 ml (sufficient for 50 tests). The polystyrene latex particles, which are coated with purified rabbit antibody to the appropriate group antigen, are suspended at a concentration of 0.5% in phosphate buffer pH 7.4 containing 0.1% sodium azide.

Extraction Enzyme

Two (ZL50/9E05-01) or eight (ZL61/1F47-01) bottles containing freeze-dried proteolytic fraction obtained from *Streptomyces griseus* cultures containing calcium chloride. When reconstituted, the working strength solution contains 0.01% Bronopol as preservative.

The reconstituted Extraction Enzyme should be stored at 2 to 8°C, when it will retain activity for at least three months after reconstitution, or until the date shown on the bottle label, whichever is the sooner. Alternatively the Enzyme may be stored in aliquots frozen at –15 to –25°C, when it will retain activity for at least six months, or until the date shown on the original bottle label, whichever is the sooner. DO NOT FREEZE AND THAW MORE THAN ONCE.

Polyvalent Positive Control[†]

One (ZL50/9E05-01) or two (ZL61/1F47-01) plastic dropper bottle(s) with a red cap containing a polyvalent extract of antigens from a representative strain of each streptococcal group A, B, C, D, F and G. The solution contains phosphate buffer pH 7.4 and 0.1% sodium azide as preservative.

WARNINGS AND PRECAUTIONS

The reagents are for *in vitro* diagnostic use only.

For professional use only.

Caution: This product contains dry natural rubber.

Please refer to the manufacturer's safety data sheet and the product labelling for information on potentially hazardous components.

HEALTH AND SAFETY INFORMATION

1. In accordance with the principles of Good Laboratory Practice it is strongly recommended that extracts at any stage of testing should be treated as potentially infectious and handled with all necessary precautions.
2. Non-disposable apparatus should be sterilised by any appropriate procedure after use, although the preferred method is to autoclave for 15 minutes at 121°C; disposables should be autoclaved or incinerated. Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated area swabbed with a standard bacterial disinfectant or 70% alcohol. Do NOT use sodium hypochlorite. Materials used to clean spills, including gloves, should be disposed of as biohazardous waste.
3. Do not pipette by mouth. Wear disposable gloves and eye protection while handling specimens and performing the assay. Wash hands thoroughly when finished.
4. The freeze-dried Extraction Enzyme contains calcium chloride which is classified per applicable European Economic Community (EEC) Directives as an irritant (Xi). The following are the appropriate Risk (R) and Safety (S) phrases.

Xi

R36	Irritating to eyes
S2	Keep out of the reach of children
S22	Do not breathe dust
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical advice
S37/39	Wear suitable gloves and eye/face protection
S46	If swallowed, seek medical advice immediately and show this container or label

5. The Latex Suspensions and Polyvalent Positive Control contain 0.1% sodium azide which is classified per applicable European Economic Community (EEC) Directives as harmful (Xn). The following are the appropriate Risk (R) and Safety (S) phrases.

Xn

R22	Harmful if swallowed
R32	Contact with acids liberates very toxic gas
S2	Keep out of the reach of children
S13	Keep away from food, drink and animal feedingstuffs
S36	Wear suitable protective clothing
S46	If swallowed, seek medical advice immediately and show this container or label

Azides can react with copper and lead used in some plumbing systems to form explosive salts. The quantities used in this kit are small; nevertheless when disposing of azide-containing materials they should be flushed away with large volumes of water.

6. When used in accordance with the principles of Good Laboratory Practice, good standards of occupational hygiene and the instructions stated in these Instructions for Use, the reagents supplied are not considered to present a hazard to health.

ANALYTICAL PRECAUTIONS

1. Do not use the reagents beyond the stated expiry date.
2. Microbiological contamination of reagents must be avoided as this may reduce the life of the product and cause erroneous results.
3. Allow all reagents and samples to come to room temperature (18 to 30°C) before use. Immediately after use return reagents to the recommended storage temperature. Latex reagents which show signs of aggregation when dispensed for the first time may have been frozen and should not be used.
4. Store Latex Reagents upright at 2 to 8°C. After prolonged storage some aggregation or drying around the top of the bottle may have occurred with the Latex Reagents. Under these circumstances the Latex Reagents should be shaken vigorously for a few seconds until resuspension is complete.
5. If the Extraction Enzyme solution becomes contaminated, as indicated by increasing turbidity during storage, it should be discarded.
6. It is important to hold the dropper bottles vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet, a drop of incorrect volume will form around the end and not at the tip; if this occurs dry the nozzle before progressing.
7. Do not touch the reaction areas on the cards.

RECONSTITUTION AND PREPARATION OF REAGENTS FOR USE

Extraction Enzyme

Reconstitute a bottle of Extraction Enzyme by adding 11 ml of sterile distilled water. Allow to stand for a few minutes with occasional swirling and inversion to aid dissolution.

SPECIMEN COLLECTION AND PREPARATION OF CULTURES

For details of specimen collection and preparation of primary cultures a standard textbook should be consulted[6]. The media used normally include blood agar and in such case the haemolytic reaction of suspected streptococcal colonies must be noted prior to attempts at grouping. Streptococci growing in mixed culture on solid primary isolation media may be reliably grouped directly if they are not overgrown by organisms such as Klebsiella, Escherichia or Pseudomonas which may non-specifically agglutinate all the latex reagents. Streptex* grouping should not be attempted on primary cultures in liquid media. When grouping from primary cultures or impure subcultures which appear to contain streptococci (if a clear result is not obtained) it is recommended that pure subcultures of suspect colonies should be made for subsequent identification by Streptex*.

Organisms of groups A, B, C, F or G are normally beta-haemolytic. If an alpha-or non-haemolytic organism appears to belong to one of these groups the species identification should be confirmed by biochemical tests[7,16]. Since enterococci are relatively resistant to penicillin, differentiation of group D organisms into enterococcal *(Enterococcus spp.)* and non-enterococcal (group D streptococci) types should be carried out by a L-pyrrolidonyl-ß-naphthylamide (PYR) hydrolysis test (Murex, Code No. LP02/8E43-01 and LP03/8E44-01) or by culture in bile esculin and 6.5% NaCl broth[6] (Figure 3). Antigen production by group D streptococci is greatly improved by addition of 0.5 to 1% glucose to the medium[14], but with blood agar the haemolytic reaction will be obscured.

PROCEDURE

MATERIALS PROVIDED

Streptex* contains sufficient material for 50 tests (ZL50/9E05-01) or 200 tests (ZL61/1F47-01).

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipette to measure and dispense 0.4 ml volumes.
- Bacteriological loop.
- Pipettes which deliver a drop volume of 40 µl.
- Water bath at 37°C.
- Glass or plastic test tubes 8 to 12 mm internal diameter, one per organism to be grouped.

TEST PROCEDURE

CAUTION: Precautions appropriate to the handling of live cultures should be taken while performing the tests.

A suggested outline scheme for grouping organisms from primary or subculture is shown in Figure 3.

Step 1	Dispense 400 µl Extraction Enzyme into an appropriately labelled test tube for each culture to be grouped.
Step 2	Using a bacteriological loop, make a light suspension of the culture in a tube of the enzyme solution. A single sweep of growth should be sufficient: it is frequently possible to obtain a result by picking as few as 5 large colonies to emulsify in the enzyme, if they adhere adequately to the loop. If the culture is not pure, it is recommended that streptococcal colonies should be picked from an area which contains as few contaminants as possible.
Step 3	Incubate the suspension at 37°C in a water bath (or in a beaker of water equilibrated to 37°C in an incubator) for a minimum of 10 minutes or any time up to 1 hour. Shake the tube after 5 minutes incubation.
Step 4	Resuspend each of the latex suspensions by shaking vigorously for a few seconds. Hold the dropper bottle vertically and dispense one drop (20 µl) of each latex suspension onto a separate circle on a Reaction Card. NOTE: It is important when using dropper bottles that they are held vertically and that the drop forms at the tip of the nozzle. If the nozzle becomes wet an incorrect volume will form around the end and not at the tip; if this occurs dry the nozzle before progressing.
Step 5	Using a pipette, place one drop (40 µl) of extract in each of the six circles on the reaction card.
Step 6	Mix the contents in each circle in turn with a mixing stick, and spread to cover the complete area of the circle. Use a separate stick for each circle and discard it for safe disposal after use.
Step 7	Rock the card gently for a maximum of one minute. The card should be held at normal reading distance (25 to 35 cm) from the eyes. Do not use a magnifying lens. The patterns obtained are clear cut and can be recognised easily under all normal lighting conditions.
Step 8	Discard the used Reaction Card for safe disposal.
Step 9	Ensure that the reagents are returned to the refrigerator, using the storage rack provided.

READING OF RESULTS

A positive result is indicated by the development of an agglutinated pattern showing clearly visible clumping of the latex particles (Figure 1).

The speed of appearance and quality of agglutination depends on the strength of the antigen extract; with a strong extract large clumps of latex particles will appear within a few seconds of mixing, but with a weak extract the reaction will take much longer to appear and the clumps of latex particles will be small.

Figure 1

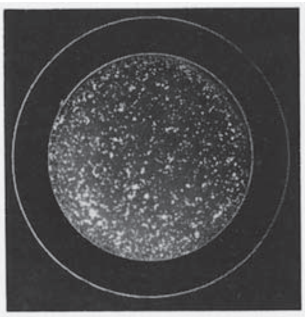
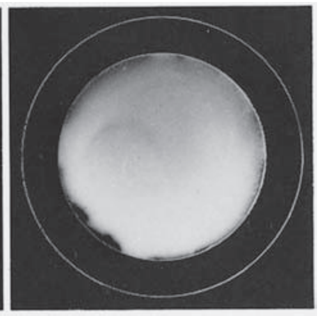


Figure 2



In a negative result the latex does not agglutinate and the milky appearance remains substantially unchanged throughout the one-minute test (Figure 2). Note, however, that faint traces of granularity may be detected in negative patterns, depending on the visual acuity of the operator.

INTERPRETATION OF RESULTS

As a general rule only beta-haemolytic streptococci provide reliable results in grouping procedures[6,8]. There are exceptions to this rule since the majority of strains of group D streptococci are either alpha-haemolytic or non-haemolytic and some strains of group B are non-haemolytic. Organisms of group D should be further classified as Enterococcus or group D streptococcus by culture in bile-esculin and 6.5% NaCl broths or PYR test[6] (Murex, Code No. LP02/8E43-01 and LP03/8E44-01); those reacting with groups A, C, F or G may, if necessary, be identified by appropriate biochemical procedures[16].

Strong rapid agglutination in only one of the six latex suspensions indicates the identity of the strain under test and delayed, weak reactions with the same extract should be ignored. Similar strength of agglutination of more than one latex suspension (but not all) indicates that the extract may contain a mixture of streptococcal groups or other bacteria containing cross-reacting antigens and further isolation procedures and/or biochemical tests should be performed. Some strains of group D streptococci have been found which appear also to possess group G antigen[1,11]. These strains will react with both group D and group G latex reagents and may be confirmed as group D if desired by the bile-esculin test[6]. For epidemiological reasons and because some of these strains possess an unusually high level of antibiotic resistance[1], it is important that they should be identified correctly.

A delayed, weak reaction in a single latex suspension usually indicates the identity of the strain under test and if possible the test should be repeated using a heavier cell suspension. When agglutination is so weak as to give rise to doubt in interpretation the test for specificity described in **Quality Control Procedures** (b) should be carried out: comparison to the two patterns will indicate the correct result.

Agglutination of all the latex reagents, which characteristically has a stringy or thread-like appearance, indicates either (a) over-inoculation of the Extraction Enzyme, in which case extraction may be repeated using a lighter suspension, or (b) contamination with an interfering organism (see **Limitations of the Procedure**) which should be eliminated by further subculture. False agglutination due to either of these causes can usually be eliminated by heating the extract in boiling water for three minutes. If none of the latex suspensions show agglutination it is likely that the culture does not belong to any of the groups covered in the test. Negative results may also be due to the use of too few organisms for extraction, particularly with group D strains – some of which yield less antigen than other groups and group F strains which have minute colonies – some of which adhere strongly to the agar surface. If a culturally-identified streptococcus does not give definite agglutination with any of the latex suspensions, it may be desirable to repeat the extraction with a larger amount of culture.

QUALITY CONTROL PROCEDURES

Initially, the laboratory should check each lot or shipment before use to verify the performance of the product using known streptococcal groups.

In normal use the performance of the test is assured by the presence of obvious agglutination in one latex suspension only, the other five suspensions showing no agglutination. This pattern of reaction may be regarded as sufficient on most occasions to demonstrate the specificity of the reagents and the efficiency of the enzymatic extraction procedure. When there is a different pattern of reaction, the following procedures are recommended:

a) **Test of the reactivity of the latex suspensions (Positive Control Procedure)**

Dispense one drop (40 µl) of Polyvalent Positive Control either in place of the test sample or in addition to it after no reaction has taken place in one minute. Mix the contents of each circle with a fresh mixing stick covering the area of the circle. After rocking the card gently for one minute, definite agglutination should occur with all the test latexes.

[†]Additional Polyvalent Positive Control is available (ZL58/1F46-01).

b) **Test for specificity of agglutination (Negative Control Procedure)**

To ensure that agglutination of a latex suspension is specific, particularly in cases of very weak agglutination or where more than one suspension is agglutinated by a single extract, repeat the positive test (or tests) simultaneously with parallel test(s) using one drop of Extraction Enzyme instead of bacterial extract. The latex suspension should not show significant agglutination in the presence of Extraction Enzyme alone and the result serves as a control for direct comparison with the pattern obtained in the presence of the bacterial extract.

c) **Test of enzyme extraction procedure**

Carry out the complete test procedure on a stock culture of known group. Occasional tests with a variety of known groups should be employed to evaluate the accuracy and efficiency of the complete test system, including the operator.

LIMITATIONS OF THE PROCEDURE

False negative results can occur if an inadequate amount of culture is used for extraction (see section **Interpretation of Results**). Some strains of *Streptococcus bovis* and *Enterococcus faecium* (group D) may not be grouped easily.

Occasional false positive results may occur with organisms from unrelated genera, for example, Klebsiella, Escherichia or Pseudomonas which may non-specifically agglutinate all latex reagents. However by examination of cultural characteristics on growth media the operator can usually eliminate these from testing. The existence of antigens common to organisms from heterologous species or genera has been demonstrated in some streptococci[2,5,15], and consequently the possibility of cross reactions of this type occurring in streptococcal grouping systems cannot be eliminated. The group D antigen is common to organisms of streptococcal groups Q, R and S[5,15].

Enterococci are relatively resistant to penicillin, but serological procedures do not differentiate between them and group D streptococci. Biochemical tests can be used for this purpose, such as PYR hydrolysis (Murex, Code No. LP02/8E43-01 and LP03/8E44-01) or growth in broth containing 6.5% NaCl. For details of the biochemical differentiation of streptococci a standard textbook should be consulted[6].

SPECIFIC PERFORMANCE CHARACTERISTICS[19]

Clinical studies were carried out in four centres in Great Britain and two in Canada on a total of 743 streptococcal cultures (663 beta-haemolytic and 80 alpha- or non-haemolytic). 290 primary cultures, 451 subcultures and 2 broth cultures were tested. The results obtained by Streptex* following both 10 minute and 60 minute extractions were compared with those found using an established reference method.

Results from 703 streptococcal cultures of groups A, B, C, D, F and G (638 beta- and 65 alpha- or non-haemolytic) are shown in Tables 1 and 2. Streptex* correctly identified 698 cultures (99%) after 10 minutes extraction and all 703 after 60 minutes. 4 beta-haemolytic cultures (1 group B, 1 group D, 1 group F and 1 group G) were missed by Streptex* after 10 minutes but correctly identified after 60 minutes extraction. One beta-haemolytic culture was grouped after 10 minutes as G but after 60 minutes extraction as B. The culture, heavily contaminated with corynebacterium spp., was not available for further study. The corynebacterium spp. from this culture did not react with Streptex*.

An additional 13 beta- and 3 alpha- or non-haemolytic cultures gave positive reactions with more than one streptococcal group with either the reference, Streptex* or both methods. These were presumed to be mixed cultures but were not available for confirmation.

Twenty four streptococcal cultures which were not grouped as A, B, C, D, F or G using the reference method did not react with Streptex*.

Table 1

Culture Identification (10 Minute Extraction)

		Streptex* Result						
		A	B	C	D	F	G	No Reaction
Established Method	A	149						
	B		214[a]				1	1
	C			64				
	D				120[b]			1
	F					14		1
	G						137	1

[a] = 197 beta- + 17 alpha- or non-haemolytic streptococcus
[b] = 72 beta- + 48 alpha- or non-haemolytic streptococcus

Table 2

Culture Identification (60 Minute Extraction)

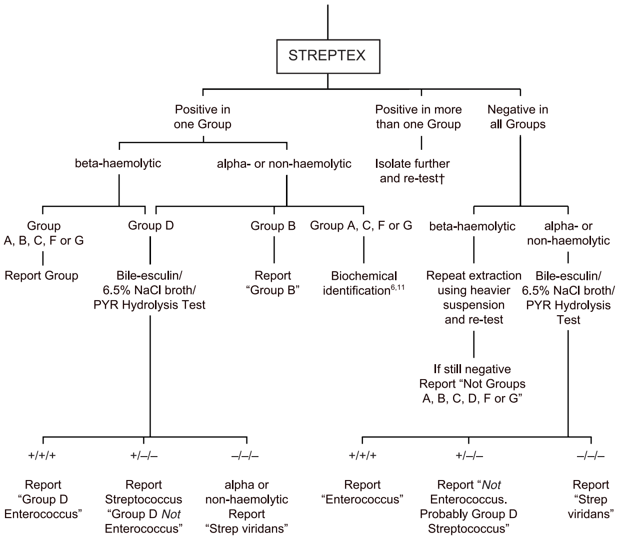
		Streptex* Result					
		A	B	C	D	F	G
Established Method	A	149					
	B		216[a]				
	C			64			
	D				121[b]		
	F					15	
	G						138

[a] = 199 beta- + 17 alpha- or non-haemolytic streptococcus
[b] = 73 beta- + 48 alpha- or non-haemolytic streptococcus

Figure 3

Suggested Scheme for Grouping Streptococci*[2,9]

Inspect streptococcal culture for type of haemolysis
and cultural characteristics.
(If alpha-haemolytic, rule out *Streptococcus pneumoniae*).
Subculture if suspected organism is scanty or overgrown.



†Rare strains have been encountered which appear to possess more than one group of antigen. After confirming the proper operation of the reagents (see **Quality Control Procedures**), problem strains should be submitted to a Reference Laboratory for identification.

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19. **Data on file, Murex.**

*trade mark.

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C07ZL61GB

March 2001

Num	EOS PIN	DATE	Results From CHCS	CULTURE RESULTS		RT-PCR RESULTS				
				NASAL WASH	THROAT SWAB	AD PAN PCR NW RESULT	AD PAN NW CT VALUES	Ad B14 NW CT Values	AD PAN TS PCR RESULT	AD PAN NW TS VALUES
1	397726	01/10/07	ADENOVIRUS		ADENOVIRUS	POS	41.34	NEGATIVE		
2	357447	01/12/07	NEGATIVE		NEGATIVE					
3	299086	01/19/07	NEGATIVE		NEGATIVE					
4	647502	01/19/07	NEGATIVE		NEGATIVE					
5	249027	01/22/07	NEGATIVE		NEGATIVE					
6	F83856	01/22/07	NO RESULT-NO REQUEST							
7	689805	01/22/07	RAPID FLU ONLY - NEG		NEGATIVE					
8	029153	01/24/07	NEGATIVE		NEGATIVE					
9	375278	01/24/07	NEGATIVE		NEGATIVE					
10	768006	01/25/07	NEGATIVE		NEGATIVE					
11	185141	01/26/07	NEGATIVE		NEGATIVE					
12	765783	01/26/07	NEGATIVE		NEGATIVE					
13	119013	01/29/07	NEGATIVE		NEGATIVE					
14	132512	01/29/07	NEGATIVE		NEGATIVE					
15	291801	01/29/07	NEGATIVE	NEGATIVE						
16	F79621	01/30/07	RAPID FLU ONLY - NEG							
17	211361	01/31/07	NEGATIVE		NEGATIVE					
18	F50388	01/31/07	RAPID FLU ONLY - NEG							
19	F01702	02/02/07	RAPID FLU ONLY - NEG							
20	F86123	02/02/07	RAPID FLU ONLY - NEG							
21	F80465	02/02/07	RAPID FLU ONLY - NEG							
22	438618	02/05/07	INFLUENZA A		INFLUENZA A					
23	986649	02/07/07	NEGATIVE		NEGATIVE					
24	F38613	02/07/07	RAPID FLU ONLY - POS A							
25	051908	02/08/07	NEGATIVE		NEGATIVE					
26	F86974	02/08/07	RAPID FLU ONLY - NEG							
27	071208	02/12/07	NEGATIVE		NEGATIVE					
28	231380	02/14/07	NEGATIVE		NEGATIVE					
29	352695	02/15/07	NEGATIVE		NEGATIVE					
30	F73301	02/15/07	RAPID FLU ONLY - NEG							
31	111640	02/16/07	NEGATIVE		NEGATIVE					
32	179375	02/20/07	NEGATIVE		NEGATIVE					
33	485280	02/20/07	NEGATIVE		NEGATIVE					
34	398481	02/20/07	ADENOVIRUS		ADENOVIRUS					
35	074345	02/22/07	NEGATIVE		NEGATIVE					
36	909764	02/22/07	NEGATIVE		NEGATIVE					
37	161139	02/22/07	NEGATIVE		NEGATIVE					
38	F18486	02/23/07	RAPID FLU ONLY - NEG							
39	599616	02/23/07	NEGATIVE		NEGATIVE					
40	593090	02/26/07	INFLUENZA A		INFLUENZA A					
41	316202	02/27/07	NEGATIVE		NEGATIVE					
42	643281	02/27/07	NEGATIVE		NEGATIVE					
43	959389	02/28/07	NEGATIVE		NEGATIVE					
44	342887	02/28/07	NEGATIVE		NEGATIVE					
45	036340	03/01/07	INFLUENZA A		INFLUENZA A					

46	F54806	03/01/07	RAPID FLU ONLY - NEG							
47	931011	03/01/07	NEGATIVE		NEGATIVE					
48	169664	03/02/07	NEGATIVE		NEGATIVE					
49	563737	03/02/07	NEGATIVE		NEGATIVE					
50	713515	03/02/07	NEGATIVE		NEGATIVE					
51	976145	03/02/07	NEGATIVE		NEGATIVE					
52	375533	03/02/07	ADENOVIRUS		ADENOVIRUS					
53	721351	03/02/07	ADENOVIRUS		ADENOVIRUS					
54	672872	03/02/07	ADENOVIRUS		ADENOVIRUS					
55	062236	03/05/07	RAPID FLU ONLY - NEG							
56	430889	03/05/07	ADENOVIRUS		ADENOVIRUS					
57	441985	03/05/07	RAPID FLU ONLY - NEG							
58	232773	03/05/07	ADENOVIRUS		ADENOVIRUS					
59	713355	03/05/07	RAPID FLU ONLY - NEG							
60	289723	03/06/07	ADENOVIRUS		ADENOVIRUS					
61	349202	03/06/07	NEGATIVE		NEGATIVE					
62	193793	03/06/07	ADENOVIRUS		ADENOVIRUS					
63	240532	03/07/07	ADENOVIRUS		ADENOVIRUS					
64	841666	03/07/07	RAPID FLU ONLY - NEG							
65	F68646	03/08/07	RAPID FLU ONLY - NEG							
66	F11641	03/08/07	RAPID FLU ONLY - NEG							
67	545415	03/09/07	RAPID FLU ONLY - NEG							
68	F22900	03/09/07	RAPID INFLUENZA A - POS							
69	165701	03/12/07	RAPID FLU ONLY - NEG							
70	F54276	03/13/07	RAPID FLU ONLY - NEG							
71	871468	03/13/07	RAPID FLU ONLY - NEG							
72	146206	03/14/07	RAPID FLU ONLY - NEG							
73	F12783	03/14/07	RAPID FLU ONLY - NEG							
74	026267	03/15/07	RAPID FLU ONLY - NEG							
75	634331	03/15/07	RAPID FLU ONLY - NEG							
76	F00843	03/15/07	RAPID FLU ONLY - NEG							
77	719997	03/15/07	RAPID FLU ONLY - NEG							
78	053991	03/16/07	RAPID FLU ONLY - NEG							
79	540628	03/16/07	RAPID FLU ONLY - NEG							
80	F27009	03/16/07	RAPID FLU ONLY - NEG							
81	F13903	03/16/07	RAPID FLU ONLY - NEG							
82	139180	03/19/07	NEGATIVE		NEGATIVE					
83	830571	03/19/07	ADENOVIRUS		ADENOVIRUS					
84	514494	03/19/07	ADENOVIRUS		ADENOVIRUS					
85	864528	03/19/07	ADENOVIRUS		ADENOVIRUS					
86	979835	03/20/07	ADENOVIRUS		ADENOVIRUS					
87	546028	03/22/07	NEGATIVE							
88	467211	03/26/07	ADENOVIRUS		ADENOVIRUS					
89	F70562	03/26/07	RAPID FLU ONLY - NEG							
90	619935	03/26/07	ADENOVIRUS		ADENOVIRUS					
91	F32018	03/27/07	RAPID FLU ONLY - NEG							
92	F04941	03/27/07	RAPID FLU ONLY - NEG							
93	365285	03/30/07	NEGATIVE		NEGATIVE					
94	557262	03/30/07	NEGATIVE		NEGATIVE					
95	944439	03/30/07	ADENOVIRUS		ADENOVIRUS					
96	979835	03/30/07	ADENOVIRUS		ADENOVIRUS					
97	513893	04/02/07	NEGATIVE		NEGATIVE					
98	425772	04/02/07	NEGATIVE		NEGATIVE					

99	F46939	04/02/07	**RAPID FLU ONLY - NEG**							
100	388342	04/03/07	**ADENOVIRUS**		**ADENOVIRUS**					
101	645504	04/03/07	**NEGATIVE**		**NEGATIVE**					
102	623847	04/03/07	**NEGATIVE**		**NEGATIVE**					
103	434041	04/04/07	**ADENOVIRUS**		**ADENOVIRUS**					
104	F20015	04/04/07	**RAPID FLU ONLY - NEG**							
105	F67791	04/04/07	**RAPID FLU ONLY - NEG**							
106	431743	04/04/07	**NO RESULT-NO REQUEST**							
107	079948	04/05/07	**ADENOVIRUS**		**ADENOVIRUS**					
108	161112	04/05/07	**NEGATIVE**		**NEGATIVE**					
109	F99142	04/05/07	**RAPID FLU ONLY - NEG**							
110	F99328	04/05/07	**RAPID FLU ONLY - NEG**							
111	634833	04/06/07	**RAPID FLU ONLY - NEG**							
112	F09166	04/06/07	**RAPID FLU ONLY - NEG**							
113	651082	04/06/07	**ADENOVIRUS**		**ADENOVIRUS**					
114	303600	04/09/07	**ADENOVIRUS**		**ADENOVIRUS**					
115	F21840	04/09/07	**RAPID FLU ONLY - NEG**							
116	896094	04/09/07	**ADENOVIRUS**		**ADENOVIRUS**					
117	078315	04/10/07	**ADENOVIRUS**		**ADENOVIRUS**					
118	438989	04/10/07	**ADENOVIRUS**		**ADENOVIRUS**					
119	592673	04/10/07	**NEGATIVE**		**NEGATIVE**					
120	876323	04/10/07	**NEGATIVE**		**NEGATIVE**					
121	393784	04/11/07	**ADENOVIRUS**		**ADENOVIRUS**					
122	031838	04/12/07	**ADENOVIRUS**		**ADENOVIRUS**					
123	F01838	04/13/07	**RAPID FLU ONLY - NEG**							
124	085764	04/16/07	**NEGATIVE**		**NEGATIVE**					
125	328143	04/16/07	**ADENOVIRUS**							
126	911799	04/16/07	**ADENOVIRUS**		**ADENOVIRUS**					
127	F45596	04/16/07	**RAPID FLU ONLY - NEG**							
128	855687	04/16/07	**ADENOVIRUS**		**ADENOVIRUS**					
129	514233	04/17/07	**ADENOVIRUS**		**ADENOVIRUS**					
130	F41007	04/17/07	**RAPID FLU ONLY - NEG**							
131	865407	04/17/07	**ADENOVIRUS**		**ADENOVIRUS**					
132	F78477	04/17/07	**CXED BY LAB - NO RESULT**							
133	224290	04/18/07	**ADENOVIRUS**		**ADENOVIRUS**					
134	F40596	04/23/07	**RAPID FLU ONLY - NEG**							
135	824901	04/23/07	**ADENOVIRUS**		**ADENOVIRUS**					
136	310204	04/24/07	**NEGATIVE**		**ADENOVIRUS**					
137	344642	04/24/07	**CXED BY LAB - NO RESULT**							
138	647711	04/30/07	**ADENOVIRUS**		**ADENOVIRUS**					
139	F15095	05/01/07	**RAPID FLU ONLY - NEG**							
140	894111	05/01/07	**ADENOVIRUS**		**ADENOVIRUS**					
141	F66912	05/03/07	**RAPID FLU ONLY - NEG**							
142	473247	05/03/07	**NEGATIVE**		**NEGATIVE**					
143	623878	05/04/07	**NEGATIVE**	**NEGATIVE**						
144	F57070	05/04/07	**NO REQUEST - NO RESULT**							
145	F69419	05/07/07	**RAPID FLU ONLY - NEG**							
146	F24954	05/07/07	**RAPID FLU ONLY - NEG**							
147	F69833	05/07/07	**RAPID FLU ONLY - NEG**							
148	601522	05/08/07	**ADENOVIRUS**		**ADENOVIRUS**					
149	928123	05/08/07	**ADENOVIRUS**		**ADENOVIRUS**					
150	F94752	05/08/07	**NO REQUEST - NO RESULT**							
151	954407	05/08/07	**RAPID FLU ONLY - NEG**							

152	691049	05/08/07	NEGATIVE							
153	F00431	05/11/07	RAPID FLU ONLY - NEG							
154	424110	05/14/07	NEGATIVE	NEGATIVE	NEGATIVE					
155	658722	05/14/07	CONTAMINATED SPECIMEN - NO RESULTS							
156	064889	05/17/07	ADENOVIRUS		ADENOVIRUS					
157	262271	05/17/07	ADENOVIRUS		ADENOVIRUS					
158	443970	05/17/07	NEGATIVE		NEGATIVE					
159	366369	05/17/07	ADENOVIRUS		ADENOVIRUS					
160	734687	05/18/07	ADENOVIRUS		ADENOVIRUS	POS	22.33	18.19		
161	178398	05/19/07	NEGATIVE		NEGATIVE					
162	353649	05/19/07	NEGATIVE		NEGATIVE					
163	933156	05/19/07	NO REQUEST - NO RESULT							
164	451763	05/19/07	ADENOVIRUS	ADENOVIRUS						
165	132534	05/21/07	ADENOVIRUS		ADENOVIRUS					
166	158670	05/21/07	NEGATIVE		NEGATIVE	NEGATIVE				
167	888275	05/21/07	ADENOVIRUS		ADENOVIRUS					
168	308668	05/21/07	ADENOVIRUS		ADENOVIRUS					
169	F31928	05/22/07	NO REQUEST - NO RESULT							
170	234622	05/23/07	ADENOVIRUS		ADENOVIRUS					
171	617689	05/23/07	ADENOVIRUS		ADENOVIRUS					
172	341206	05/24/07	ADENOVIRUS		ADENOVIRUS	POS	27.33	22.51		
173	F11743	05/24/07	ADENOVIRUS		NEGATIVE					
174	945843	05/24/07	ADENOVIRUS		ADENOVIRUS					
175	889811	05/25/07	ADENOVIRUS	ADENOVIRUS		POS	32.38	26.43		
176	043281	05/25/07	NEGATIVE		NEGATIVE					
177	584118	05/25/07	ADENOVIRUS	ADENOVIRUS						
178	675741	05/25/07	CXED BY LAB - NO RESULT							
179	760596	05/25/07	NEGATIVE		NEGATIVE					
180	050396	05/26/07	NEGATIVE		NEGATIVE					
181	119732	05/26/07	ADENOVIRUS		ADENOVIRUS					
182	936014	05/26/07	ADENOVIRUS		ADENOVIRUS					
183	671489	05/26/07	ADENOVIRUS		ADENOVIRUS					
184	179485	05/28/07	ADENOVIRUS		ADENOVIRUS					
185	523000	05/28/07	ADENOVIRUS		ADENOVIRUS					
186	704291	05/28/07	NEGATIVE		NEGATIVE					
187	821631	05/28/07	NEGATIVE		NEGATIVE					
188	322551	05/28/07	ADENOVIRUS		ADENOVIRUS					
189	057873	05/30/07	ADENOVIRUS	ADENOVIRUS	ADENOVIRUS	POS	37.01	25.29	POS	25.85
190	203938	05/30/07	NEGATIVE		NEGATIVE	NEG			NEG	
191	335700	05/30/07	ADENOVIRUS	ADENOVIRUS	ADENOVIRUS					
192	695884	05/30/07	ADENOVIRUS	ADENOVIRUS	ADENOVIRUS	POS	30.46	25.09	POS	21.30
193	864173	05/30/07	NEGATIVE	NEGATIVE	NEGATIVE	NEG			NEG	
194	090213	05/31/07	ADENOVIRUS		ADENOVIRUS	POS	30.47	23.57	POS	30.95
195	141308	05/31/07	ADENOVIRUS		ADENOVIRUS	POS	37.76	31.73	POS	28.53
196	216950	05/31/07	ADENOVIRUS		ADENOVIRUS				POS	36.13
197	484229	05/31/07	NEGATIVE		NEGATIVE	NEG			NEG	
198	641366	05/31/07	ADENOVIRUS		ADENOVIRUS	POS	37.76	30.72	POS	32.46
199	934211	05/31/07	ADENOVIRUS		ADENOVIRUS	POS	27.74	22.68		
200	741131	05/31/07	ADENOVIRUS		ADENOVIRUS	POS	31.93	26.23		
201	315450	06/01/07	ADENOVIRUS		ADENOVIRUS	POS	31.50	26.15		
202	777807	06/01/07	NEGATIVE		NEGATIVE	NEGATIVE				
203	781526	06/02/07	ADENOVIRUS		ADENOVIRUS	POS	36.01	30.48		
204	274770	06/04/07	ADENOVIRUS		ADENOVIRUS	POS	34.31	29.96		

205	757264	06/04/07	NEGATIVE		NEGATIVE	NEG			NEG	
206	805608	06/04/07	ADENOVIRUS		ADENOVIRUS	POS	24.17	19.50		
207	502117	06/05/07	ADENOVIRUS		ADENOVIRUS	NEG			NEG	
208	656871	05/31/07	NEGATIVE		NEGATIVE	NEG			NEG	
209	748269	06/02/07	NEGATIVE		NEGATIVE	NEG			NEG	
210	699998	06/02/07	NEGATIVE		NEGATIVE	POS	41.06	36.45	NEG	
211	008080	06/02/07	ADENOVIRUS	ADENOVIRUS	ADENOVIRUS	NEG			NEG	
212	891737	06/02/07	NEGATIVE		NEGATIVE	NEG			NEG	
213	079386	06/05/07	NEGATIVE		NEGATIVE	NEG			NEG	
214	784232	06/05/07	ADENOVIRUS		ADENOVIRUS	POS	33.20	27.62	NEG	
215	F73977	05/28/07	ADENOVIRUS	ADENOVIRUS		POS	37.15	31.67	POS	42.68
216	F81502	05/29/07	ADENOVIRUS	ADENOVIRUS		POS	34.49	29.32		
217	F50534	05/29/07	ADENOVIRUS	ADENOVIRUS		POS	28.82	23.71		
218	F30273	05/29/07	ADENOVIRUS		ADENOVIRUS	POS	34.25	29.41		
219	F65543	05/30/07	ADENOVIRUS		ADENOVIRUS	NEG			POS	23.02
220	F71191	06/01/07	COXSACKIE - B		COXSACKIE - B	NEG			NEG	
221	F04270	06/04/07	CXED BY LAB - NO RESULT			NEG		37.12	POS	35.05
222	F67463	06/04/07	CXED BY LAB - NO RESULT							
223	F74763	06/04/07	CXED BY LAB - NO RESULT			NEG		36.08	POS	28.92
224	F83049	06/05/07	CXED BY LAB - NO RESULT			POS	35.67	35.71	POS	32.08
225	F56071	06/06/07	ADENOVIRUS		ADENOVIRUS	POS	33.89	28.21	POS	25.35
226	168819	06/07/07	NEGATIVE		NEGATIVE					
227	945400	06/07/07	ADENOVIRUS		ADENOVIRUS	POS	28.78	24.01	POS	32.79
228	F80750	06/07/07	ADENOVIRUS		ADENOVIRUS	POS	31.10	26.58	POS	32.96
229	F50264	06/07/07	ADENOVIRUS		ADENOVIRUS	POS	34.61	30.17	POS	29.15
230	F63130	06/08/07	ADENOVIRUS		ADENOVIRUS	POS	36.27	31.48		
231	516659	06/08/07	ADENOVIRUS		ADENOVIRUS	POS	28.82	24.13	POS	32.29
232	581944	06/09/07	ADENOVIRUS		ADENOVIRUS	POS	31.57	25.44		
233	037359	06/11/07	NEGATIVE		NEGATIVE	NEG				
234	905217	06/11/07	NEGATIVE		NEGATIVE	NEG			NEG	
235	F15310	06/11/07	ADENOVIRUS		ADENOVIRUS	POS	27.78	22.13		
236	864401	06/12/07	ADENOVIRUS		ADENOVIRUS	POS	37.42	32.91		
237	898035	06/12/07	NEGATIVE		NEGATIVE	NEGATIVE				
238	F65041	06/12/07	ADENOVIRUS		ADENOVIRUS	POS	27.40	21.93		
239	917533	6/13/2007	NEGATIVE		NEGATIVE	NEGATIVE				
240	173240	6/13/2007	NEGATIVE		NEGATIVE					
241	418678	6/13/2007	NEGATIVE		NEGATIVE	NEGATIVE				
242	093044	6/13/2007	NEGATIVE		NEGATIVE	NEGATIVE				
243	652746	6/18/2007	NEGATIVE		NEGATIVE	NEGATIVE				
244	F52802	6/19/2007	NEGATIVE		NEGATIVE	NEG				
245	256584	6/20/07	ADENOVIRUS		ADENOVIRUS	POS	35.57	29.72		
246	384145	6/20/07	NEGATIVE			NEG				
247	078090	6/20/07	NEGATIVE			NEG				
248	708124	6/25/07	NEGATIVE			NEG				
249	639487	6/25/07	ADENOVIRUS		ADENOVIRUS	POS	28.67	23.85		
250	925762	6/25/07	ADENOVIRUS		ADENOVIRUS	POS	31.49	26.41		
251	179180	6/26/07	NEGATIVE			NEG				
252	292337	6/26/07	ADENOVIRUS		ADENOVIRUS	POS	26.73	21.87		
253	501476	6/26/07	NEGATIVE			NEG				
234C	905217	6/26/07	NEGATIVE			NEG				
254	112525	6/27/07	NEGATIVE		NEGATIVE	NEG				
255	194389	6/27/07	ADENOVIRUS		ADENOVIRUS	NEG				
256	F91227	6/27/07	NEGATIVE			NEG				

257	842213	6/28/07	NEGATIVE		NEGATIVE	POS	30.22	23.01		
242C	093044	6/28/07	NEGATIVE			POS	NEG	39.58		
258	274724	6/29/07	ADENOVIRUS		ADENOVIRUS	POS	38.34	27.54		
246C	384145	7/2/07	Unable to collect F/U enrolled 6/20/07, grad. 7/5							
241C	418678	7/2/07	NEGATIVE		NEGATIVE	NEG				
259	807674	7/3/07	NEGATIVE		NEGATIVE	NEG				
260	055167	7/3/07	NEGATIVE		NEGATIVE	NEG				
261	319372	7/3/07	NEGATIVE		NEGATIVE					
243C	652746	7/3/07	Unable to collect F/U, enrolled 6/18/07							
262	F86355	7/9/2007	NO REQUEST - NO RESULT							
263	136741	7/9/2007	ADENOVIRUS		ADENOVIRUS	POS	30.64	24.98		
264	529912	7/9/2007	ADENOVIRUS		ADENOVIRUS	POS	29.50	21.74		
265	681723	7/10/07	ADENOVIRUS		ADENOVIRUS	POS	33.03	25.13		
266	891506	7/10/07	NEGATIVE		NEGATIVE					
245C	256584	7/10/07	NEGATIVE		NEGATIVE					
247C	078090	7/10/07	NEGATIVE		NEGATIVE					
267	601995	7/11/07	ADENOVIRUS		ADENOVIRUS	POS	27.05	21.68		
268	049770	7/11/07	NEGATIVE		NEGATIVE	NEG				
252C	292337	7/11/07	NEGATIVE		NEGATIVE	NEG				
255C	194389	7/11/07	NEGATIVE		NEGATIVE	NEG				
269	803825	7/12/07	ADENOVIRUS		ADENOVIRUS	POS	32.28	26.86		
270	879421	7/12/07	ADENOVIRUS		ADENOVIRUS	POS	37.20	29.68		
253C	501476	7/12/07	NEGATIVE		NEGATIVE	NEG				
271	056875	7/13/07	NEGATIVE		NEGATIVE	NEG				
272	797995	7/13/07	ADENOVIRUS	ADENOVIRUS		POS	28.95	22.53		
251C	179180	7/13/07	NEGATIVE			NEG				
273	738853	7/16/07	NEGATIVE			NEG				
274	259699	7/17/07	NEGATIVE			NEG				
275	851803	7/17/07	ADENOVIRUS		ADENOVIRUS	POS	31.38	24.17		
276	F42904	7/17/07	ADENOVIRUS		ADENOVIRUS	POS	28.49	21.85		
277	F64062	7/17/07	ADENOVIRUS		ADENOVIRUS	POS	37.63	31.17		
278	904213	7/18/07	NEGATIVE			NEG				
279	599788	7/18/07	ADENOVIRUS		ADENOVIRUS	POS	25.48	19.82		
254C	112525	7/18/07	Unable to collect F/U, enrolled 6/27/07							
280	923041	7/20/07	NEGATIVE			NEG				
281	981862	7/20/07	ADENOVIRUS	ADENOVIRUS		POS	30.42	25.60		
259C	055167	7/20/07	ADENOVIRUS	ADENOVIRUS		POS	36.91	29.77		
282	F29414	7/23/07	ADENOVIRUS	ADENOVIRUS		POS	31.45	25.56		
283	F32274	7/23/07	NEGATIVE			NEG				
284	732642	7/24/07	ADENOVIRUS	ADENOVIRUS		POS	31.59	23.32		
259C	807674	7/24/07	Unable to collect F/U, enrolled 7/03/07							
261C	319372	7/24/07	Unable to collect F/U, enrolled 7/03/07							
285	441670	7/25/07	ADENOVIRUS	ADENOVIRUS		POS	31.85	25.35		
286	082531	7/26/07	NEGATIVE			NEG				
287	639141	7/26/07	ADENOVIRUS	ADENOVIRUS		POS	28.35	21.89		
288	096625	7/26/07	ADENOVIRUS	ADENOVIRUS		POS	25.79	19.64		
289	501149	7/26/07	ADENOVIRUS	ADENOVIRUS		POS	21.82	15.82		
290	183386	7/27/07	NEG	NEG		POS	NEG	37.31		
291	057857	7/27/07	NEG	NEG		NEG				
292	069362	7/28/07	ADENOVIRUS	ADENOVIRUS		POS	28.72	20.72		
293	863506	7/28/07	ADENOVIRUS	ADENOVIRUS		POS	36.24	29.09		
294	140706	7/28/07	ADENOVIRUS	ADENOVIRUS		POS	31.62	25.35		
295	482174	7/28/07	ADENOVIRUS	ADENOVIRUS		POS	NEG	34.17		

296	708487	7/28/07	**NEG**			**NEG**				
297	809547	7/28/07	**NEG**			**NEG**				

Ad B14 TS CT Values	NHRC SUBTYPE	RAPID ADENO TEST	Miss Results & Comments		
	Ad B 3				
			TC - POSITIVE BETA STREP GROUP A		
			TC - POSITIVE BETA STREP GROUP A		
			TC - POSITIVE BETA STREP GROUP A		

	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad B 14				
			TC - BETA STREP NOT GROUP A		
	Ad B 14				
	Ad B 14				
	Ad E 4a				
	Ad E 4a				
	Ad 3 & 4a				
	Ad E 4a				

	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad B 14				
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	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad E 4a				
	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad B 14				
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	Ad B 14				
	Ad B 14				
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	Ad B 3				
	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad E 4a				
	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad B 14				
	Ad E 4a				
	Ad B 14	POS			
	Ad B 14	POS			
	Ad B 14	NEG			
	Ad B 14	NEG			
		NEG			
		NEG			
		POS			
		NEG			
	Ad B 14	NEG			
	Ad B 14	POS			
	Ad B 14	NEG			
	Ad B 14	POS			
	Ad B 14	POS			
		POS			
	Ad B 14	NEG			
	Ad B 14	NEG			
	Ad B 14	NEG			
25.29	**Ad B 14**	NEG			
	Ad B 14	NEG			
	Ad B 14	NEG			
25.09	**Ad B 14**	POS			
		NEG			
23.57	**Ad B 14**	NEG			
31.73	**Ad B 14**	POS			
		NEG			
	Ad B 14	NEG			
30.72	**Ad B 14**	NEG			
	Ad B 14	POS			
	Ad B 14	POS			
		NEG			
		NEG			
		NEG			
	Ad B 14	POS			

		NEG			
	Ad B 14	POS			
	Ad B 14	NEG			
		NEG			
		NEG			
36.45	Ad B 14	NEG			
		NEG			
		NEG			
		NEG			
27.62	Ad B 14	NEG			
31.67	Ad B 14	POS			
	Ad B 14	NEG			
	Ad B 14	POS			
29.41	Ad B 14	POS			
	Ad B 14	POS			
		NEG			
37.12	Ad B 14	NEG			
		POS			
36.08	Ad B 14	NEG			
35.71	Ad B 14	NEG			
28.21	Ad B 14	POS			
		NEG			
24.01	Ad B 14	NEG			
26.58	Ad B 14	POS			
30.17	Ad B 14	POS			
		NEG			
24.13	Ad B 14	NEG			
	Ad B 14	POS			
		NEG			
		NEG			
	Ad B 14	POS			
	Ad B 14	NEG			
		NEG			
		POS			
		NEG			
		NEG			
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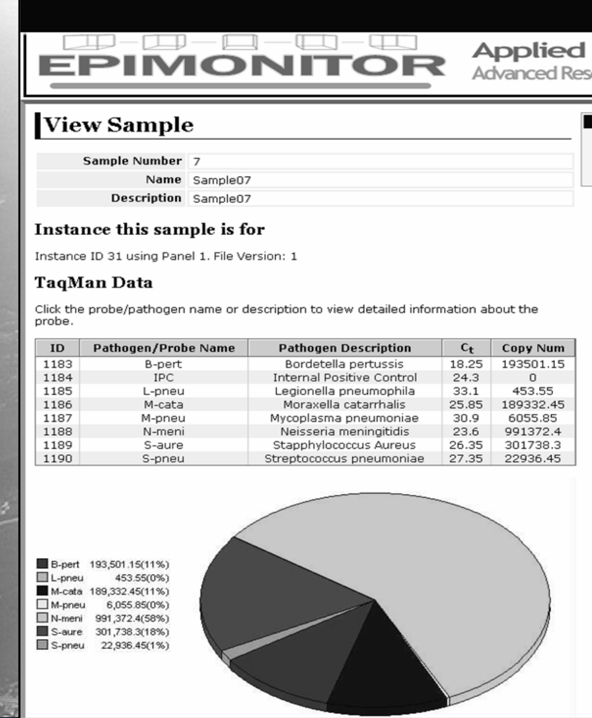
		NEG			
		NEG			
		NEG			
		NEG			
		NEG			
		NEG			
		NEG			
		POS			
		POS			
		NEG			
		POS			
		POS			
		follow-up convalescent visit, enrolled 6/11/07			
		NEG			
		NEG			
		NEG			

		POS	
		follow-up convalescent visit, enrolled 6/13/07	
		NEG	
		N/A	
		follow-up convalescent visit, enrolled 6/13/07	
		NEG	
		NOT NOTED	
		NEG	
		N/A	
		NEG	
		NEG	
		POS	
		NEG	
		NEG	
		follow-up convalescent visit, enrolled 6/20/07	
		follow-up convalescent visit, enrolled 6/20/07	
		POS	
		NEG	
		follow-up convalescent visit, enrolled 6/20/07	
		follow-up convalescent visit, enrolled 6/20/07	
		NEG	
		NEG	
		follow-up convalescent visit, enrolled 6/26/07	
		NEG	
		POS	
		follow-up convalescent visit, enrolled 6/26/07	
		NEG	
		NEG	
		POS	
		POS	
		NEG	
		POS	
		NEG	
		NEG	
		NEG	
		follow-up convalescent visit, enrolled 7/3/07	
		NEG	
		NEG	
		POS	
		NEG	
		NEG	
		POS	
		POS	
		POS	
		NEG	TC - POSITIVE BETA STREP GROUP A
		NEG	
		POS	
		POS	
		POS	
		POS	

		POS
		POS



Applied Biosystems

Nasal Wash and Throat Swabs from culture positive clinical samples

March 20, 2007

Purpose of Experiment

- Determine in a small sample set if TLDA and pMD are sensitive enough to detect pathogens on EOS ACTD Tier 1 list
- This is not an attempt to determine clinically relevant LOD since pathogen concentration was not known.
- Use samples from USAF Lackland Airforce base, ADL
- Samples were
  - symptomatic + culture positive, or
  - asymptomatic: “Normal”

Presentation Outline

- Data on 24 matched nasal wash and swab samples from culture positive Flu A patients
  - TLDA data from AB
  - TLDA data from DoH
  - pMD data (4 nasal wash samples)

- Data from 56 "blind" culture positive samples
  - TLDA data from AB
  - TLDA data from DoH
  - pMD data (12 samples)

Data on 24 Matched Positive Samples

- Data on 24 matched nasal wash and swab samples from culture positive Flu A patients
  - TLDA data from AB
  - TLDA data from DoH
  - pMD data (4 nasal wash samples)

Applied Biosystems

Experimental Outline

➢ Received 24 characterized samples from Lackland Air Force Base in the form of matched (same patient) Nasal Wash and expressed Throat Swabs

- Influenza A was expected

➢ 150ul of each sample was processed through the IDIS Purification Protocol at AB.

- Half of the resulting purified nucleic acid was shipped to Florida Department of Health Lab in Miami and the other half remained at AB

➢ Nucleic Acid samples were analyzed on the SGURv1.0 TLDA cards at both sites in parallel

➢ 4 Nasal Wash/Throat Swab samples were also processed through the pMD Breadboard Sample Preparations System and analyzed on a pMD Card

25 YEARS ADVANCING SCIENCE

24 Nasal Wash Samples purified through IDIS and run on SGURv1.0 TLDA (Foster City)

Applied Biosystems

		Internal Positive Control	Influenza A	Influenza A-H1	Influenza A-H3	Influenza A-H5a	Influenza A-H5b	Influenza B	SARS.1	RSV	Human Adenovirus Panel	Rnase P	Human Adenovirus 4	Human PIV 1	Human PIV 2	Human PIV 3	Human Metapnuemovirus	Streptococcus pneumoniae	Streptococcus pyogenes	Mycoplasma pneumoniae	Chlamydia pneumoniae	Bordetella pertussis 1	Bortetella pertussis 2	Legionella pneumophila	XenoRNA Control
	F04274 (Nasal Wash)	27.17	27.16	-	30.87	-	-	-	-	-	-	31.88	-	-	-	-	-	-	-	-	-	-	-	-	31.13
		27.16	27.22	-	30.33	-	-	-	-	-	-	31.22	-	-	-	-	-	-	-	-	-	-	-	-	30.71
	F14600 (Nasal Wash)	27.23	33.93	-	35.72	-	-	-	-	-	-	32.36	-	-	-	-	-	-	-	-	-	-	-	-	30.44
		27.19	35.24	-	36.55	-	-	-	-	-	-	35.11	37.34	-	-	-	-	-	-	-	-	-	-	-	30.03
	F15697 (Nasal Wash)	26.90	31.64	-	35.53	-	-	-	-	-	-	35.84	-	-	-	-	-	-	-	-	-	-	-	-	29.76
		26.92	30.49	-	34.62	-	-	-	-	-	-	34.29	-	-	-	-	-	-	-	-	-	-	-	-	30.00
	F19887 (Nasal Wash)	27.25	27.42	-	31.22	-	-	-	-	-	-	30.59	-	-	-	-	-	-	-	-	-	-	-	-	29.79
		27.12	27.35	-	31.37	-	-	-	-	-	-	30.51	-	-	-	-	-	-	-	-	-	-	-	-	29.73
30ul loaded	F20856 (Nasal Wash) 30ul	26.97	34.20	-	-	-	-	-	-	-	-	33.45	-	-	-	-	-	-	-	-	-	-	-	-	29.46
		26.84	35.55	-	-	-	-	-	-	-	-	33.03	-	-	-	-	-	-	-	-	-	-	-	-	29.25
	F36027 (Nasal Wash)	27.18	32.83	-	36.70	-	-	-	-	-	-	32.05	-	-	-	-	-	-	-	-	-	-	-	-	30.54
		27.20	33.24	-	37.06	-	-	-	-	-	-	32.63	-	-	-	-	-	-	-	-	-	-	-	-	30.25
	F36978 (Nasal Wash)	27.05	29.67	-	33.72	-	-	-	-	-	-	30.96	-	-	-	-	-	-	-	-	-	-	-	-	30.27
		27.37	29.26	-	33.63	-	-	-	-	-	-	31.44	-	-	-	-	-	-	-	-	-	-	-	-	30.18
	F37607 (Nasal Wash)	27.12	27.35	-	30.68	-	-	-	-	-	-	30.29	-	-	-	-	-	-	-	-	-	-	-	-	30.62
		27.29	27.56	-	30.13	-	-	-	-	-	-	30.11	-	-	-	-	-	-	-	-	-	-	-	-	30.43
30ul loaded	F40239 (Nasal Wash) 30ul	26.80	-	-	-	-	-	-	-	-	35.22	32.88	-	-	-	-	-	-	-	-	-	31.76	-	-	30.68
		26.91	35.90	-	-	-	-	-	-	-	-	32.09	-	-	-	-	-	-	-	-	-	-	-	-	30.79
	F40366 (Nasal Wash)	27.01	24.12	-	28.14	-	-	-	-	-	-	32.12	-	-	-	-	-	-	-	-	-	-	-	-	30.77
		27.08	24.11	-	28.10	-	-	-	-	-	-	31.06	-	-	-	-	-	-	-	-	-	-	-	-	30.28
	F42651 (Nasal Wash)	27.11	27.30	-	31.19	-	-	-	-	-	-	33.04	-	-	-	-	-	-	-	-	-	-	-	-	30.29
		27.23	27.42	-	30.94	-	-	-	-	-	-	31.97	-	-	-	-	-	-	-	-	-	-	-	-	30.23
30ul loaded	F43417 (Nasal Wash) 30ul	28.03	31.08	-	-	-	-	-	-	-	-	30.15	-	-	-	-	-	-	-	-	-	-	-	-	31.88
		28.15	30.41	-	-	-	-	-	-	-	-	29.98	-	-	-	-	-	-	-	-	-	-	-	-	30.52
	NTC 1	27.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		27.71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NTC 2	27.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		27.80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

25 YEARS ADVANCING SCIENCE

24 Nasal Wash Samples purified through IDIS and run on SGURv1.0 TLDA (Foster City)

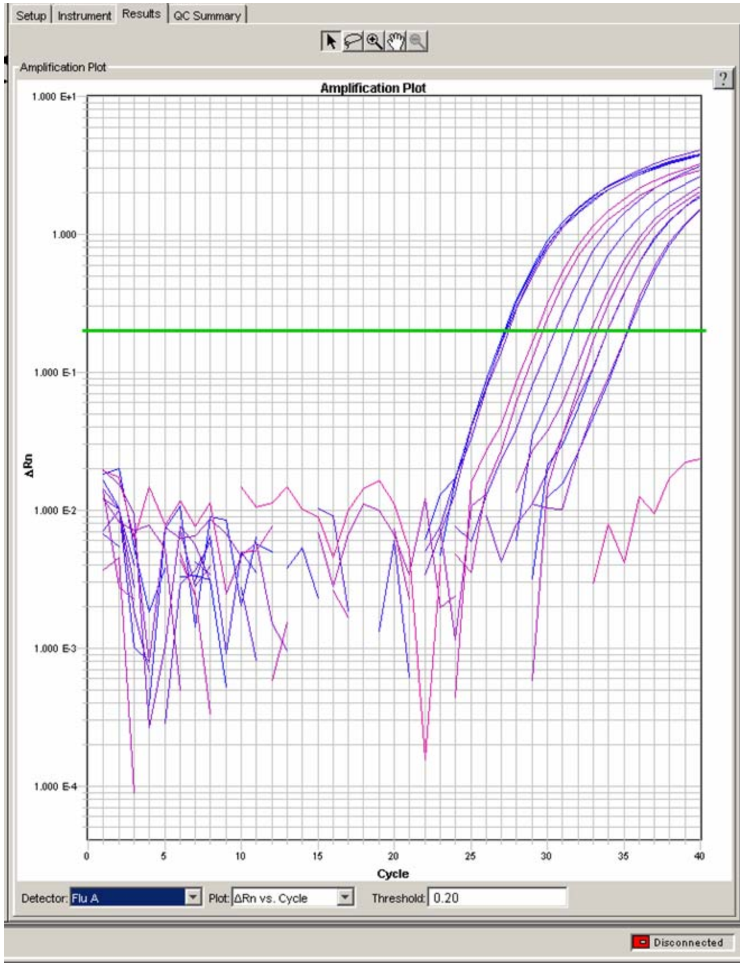
Applied Biosystems

		Internal Positive Control	Influenza A	Influenza A-H1	Influenza A-H3	Influenza A-H5a	Influenza A-H5b	Influenza B	SARS.1	RSV	Human Adenovirus Panel	Rnase P	Human Adenovirus 4	Human PIV 1	Human PIV 2	Human PIV 3	Human Metapnuemovirus	Streptococcus pneumoniae	Streptococcus pyogenes	Mycoplasma pneumoniae	Chlamydia pneumoniae	Bordetella pertussis 1	Bortetella pertussis 2	Legionella pneumophila	XenoRNA Control
	F43541 (Nasal Wash)	27.40	25.07	-	29.66	-	-	-	-	-	-	30.29	-	-	-	-	-	-	-	-	-	-	-	-	29.94
		27.21	25.69	-	29.99	-	-	-	-	-	-	30.78	-	-	-	-	-	-	-	-	-	-	-	-	30.18
	F43632 (Nasal Wash)	27.10	29.75	-	32.92	-	-	-	-	-	-	30.04	-	-	-	-	-	-	-	-	-	-	-	-	30.77
		27.74	29.53	-	33.00	-	-	-	-	-	-	30.16	-	-	-	-	-	-	-	-	-	-	-	-	30.08
30ul loaded	F43789 (Nasal Wash) 30ul	26.66	35.65	-	-	-	-	-	-	-	-	33.35	-	-	-	-	-	-	-	-	-	-	-	-	28.57
		26.67	35.29	-	-	-	-	-	-	-	-	34.07	-	-	-	-	-	-	-	-	-	-	-	-	28.99
	F47181 (Nasal Wash)	26.78	26.30	-	30.26	-	-	-	-	-	-	29.41	-	-	-	-	-	-	-	-	-	-	-	-	30.39
		27.07	26.63	-	30.32	-	-	-	-	-	-	29.87	-	-	-	-	-	-	-	-	-	-	-	-	29.90
30ul loaded	F47994 (Nasal Wash) 30ul	27.24	31.77	-	35.23	-	-	-	-	-	-	30.50	-	-	-	-	-	-	-	-	-	-	-	-	28.82
		26.81	31.99	-	34.76	-	-	-	-	-	-	30.29	-	-	-	-	-	-	-	-	-	-	-	-	28.65
30ul loaded	F49098 (Nasal Wash) 30ul	26.64	31.82	-	38.71	-	-	-	-	-	-	30.96	-	-	-	-	-	-	-	-	-	-	-	-	28.70
		26.88	31.56	-	35.14	-	-	-	-	-	-	31.23	-	-	-	-	-	-	-	-	-	-	-	-	28.58
	F52052 (Nasal Wash)	26.68	31.39	-	34.40	-	-	-	-	-	-	30.30	-	-	-	-	-	-	-	-	-	-	-	-	29.77
		26.78	30.63	-	34.40	-	-	-	-	-	-	29.82	-	-	-	-	-	-	-	-	-	-	-	-	29.99
	F55355 (Nasal Wash)	27.24	26.31	-	31.58	-	-	-	-	-	-	30.11	-	-	-	-	-	-	-	-	-	-	-	-	30.25
		26.95	26.98	-	31.34	-	-	-	-	-	-	29.64	-	-	-	-	-	-	-	-	-	-	-	-	29.99
30ul loaded	F62888 (Nasal Wash) 30ul	26.85	32.27	-	37.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	28.98
		26.82	32.07	-	34.84	-	-	-	-	-	-	34.78	-	-	-	-	-	-	-	-	-	-	-	-	29.22
	F65790 (Nasal Wash)	26.97	25.15	-	29.39	-	-	-	-	-	-	32.28	-	-	-	-	-	-	-	-	-	-	-	-	30.49
		26.97	24.91	-	28.60	-	-	-	-	-	-	31.55	-	-	-	-	-	-	-	-	-	-	-	-	30.20
	F68876 (Nasal Wash)	26.96	27.00	-	31.10	-	-	-	-	-	-	33.24	-	-	-	-	-	-	-	-	-	-	-	-	29.95
		27.21	27.19	-	30.63	-	-	-	-	-	-	32.75	-	-	-	-	-	-	-	-	-	-	-	-	29.98
	f75301 (Nasal Wash)	26.97	30.02	-	34.08	-	-	-	-	-	-	31.60	-	-	-	-	-	-	-	-	-	-	-	-	29.99
		26.86	30.02	-	34.43	-	-	-	-	-	-	31.14	-	-	-	-	-	-	-	-	-	-	-	-	29.83
	NTC 3	27.06	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		27.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	NTC 4	27.16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
		27.55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

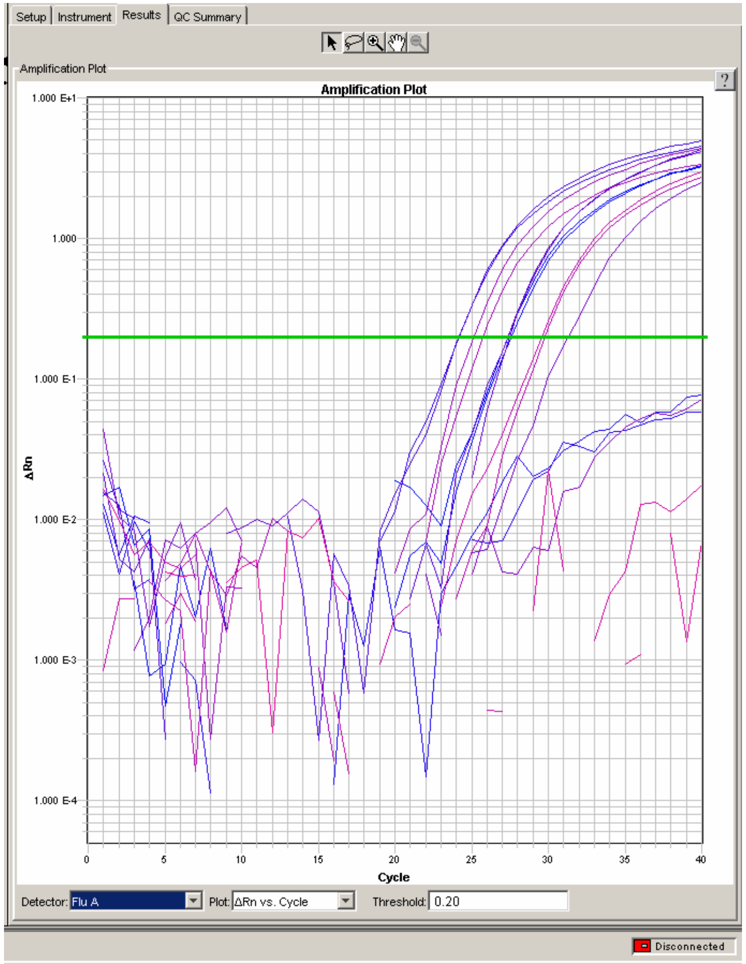
APPLIED BIOSYSTEMS PROPRIETARY INFORMATION COVERED BY CONFIDENTIALITY AND NONDISCLOSURE BETWEEN APPLIED BIOSYSTEMS AND USAF

25 YEARS ADVANCING SCIENCE

Real-time PCR results for 24 Samples run on SGUR v1.0 TLDA



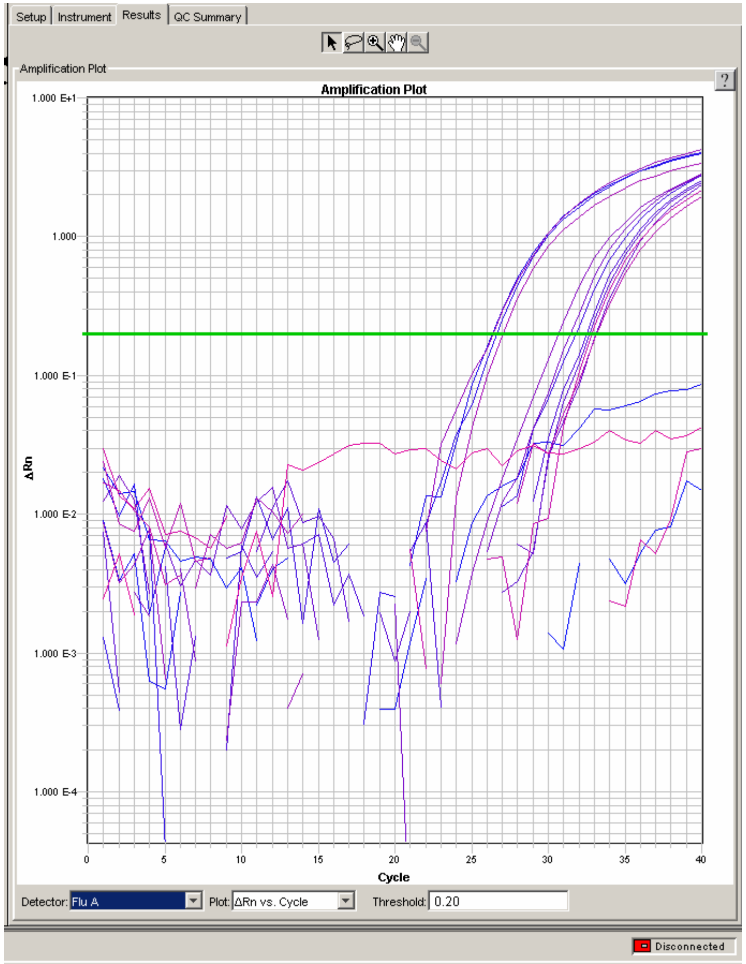
Samples 1-7



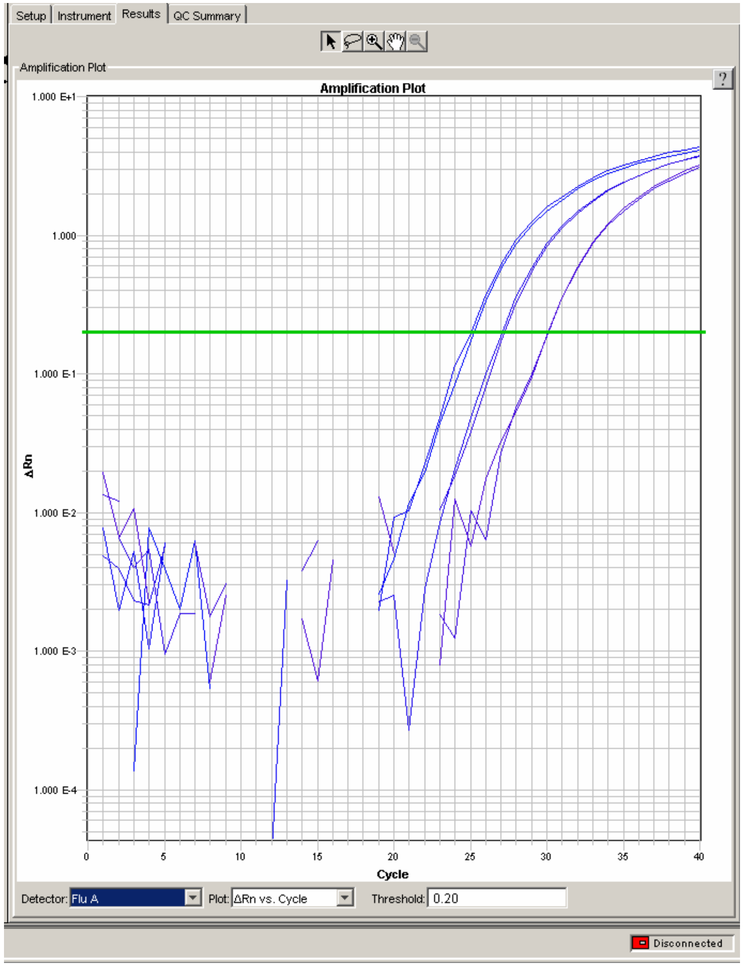
Samples 8-14

Real-time PCR results for 24 Samples run on SGUR v1.0 TLDA

Applied Biosystems



Samples 15-21



Samples 22-24

25 YEARS ADVANCING SCIENCE

Comparison of TLDA and Culture data

Applied Biosystems

30ul	Influenza A (AB-Foster City) Nasal Wash	Influenza A (AB-Foster City) Throat Swab	Influenza A (FL-DOH) Nasal Wash	Influenza A (FL-DOH) Throat Swab	USAF Tissue Culture Test Result Nasal Wash	USAF Tissue Culture Test Result Throat Swab
F04274	27.16	32.93	26.85	33.93	+	+
	27.22	-	26.88	34.27		
F14600	33.93	33.18	35.48	-	+	+
	35.24	33.64	35.13	33.38		
F15697	31.64	-	31.75	-	+	+
	30.49	-	31.95	34.75		
F19887	27.42	36.61	30.13	34.81	+	+
	27.35	35.90	30.74	35.38		
F20856	34.20	36.97	33.56	35.70	+	+
	35.55	34.53	35.82	-		
F36027	32.83	33.08	32.39	33.32	+	+
	33.24	33.01	32.46	33.99		
F36978	29.67	35.79	28.87	-	+	-
	29.26	35.47	28.42	-		
F37607	27.35	34.72	26.65	36.10	+	-
	27.56	35.64	27.01	-		
F40239	-	-	-	-	+	-
	35.90	-	-	-		
F40366	24.12	32.72	24.50	33.45	+	-
	24.11	32.51	24.54	33.21		
F42651	27.30	35.72	27.04	34.61	+	-
	27.42	34.52	27.37	35.72		
F43417	31.08	-	30.60	-	+	-
	30.41	-	31.39	-		
F43417	31.08	-	30.60	-	+	-
	30.41	-	31.39	-		
F43541	25.07	-	25.32	-	+	-
	25.69	-	25.22	-		
F43632	29.75	35.82	29.27	-	+	-
	29.53	-	28.98	-		
F43789	35.65	34.30	-	33.41	+	-
	35.29	33.31	-	35.44		
F47181	26.30	35.92	27.47	35.04	+	-
	26.63	35.55	27.47	-		
F47994	31.77	-	33.82	-	+	-
	31.99	-	33.90	-		
F49098	31.82	-	33.80	-	+	-
	31.56	-	35.57	-		
F52052	31.39	-	29.20	-	+	-
	30.63	-	29.07	-		
F55355	26.31	-	28.60	-	+	-
	26.98	-	28.29	-		
F62888	32.27	34.00	34.36	-	+	-
	32.07	33.36	36.52	-		
F65790	25.15	-	24.95	-	+	-
	24.91	-	25.75	-		
F68876	27.00	31.27	27.32	32.85	+	-
	27.19	31.93	27.52	34.11		

25 YEARS ADVANCING SCIENCE

4 Nasal Wash Samples - IDIS/TLDA compared to pMD

Applied Biosystems

IDIS-SGURv1.0 TLDA

Sample	Internal Positive Control	Influenza A	Influenza A-H1	Influenza A-H3	Influenza A-H5a	Influenza A-H5b	Influenza B	SARS.1	RSV	Human Adenovirus Panel	Rnase P	Human Adenovirus 4	Human PIV 1	Human PIV 2	Human PIV 3	Human Metapnuemovirus	Streptococcus pneumoniae	Streptococcus pyogenes	Mycoplasma pneumoniae	Chlamydia pneumoniae	Bordetella pertussis 1	Bortetella pertussis 2	Legionella pneumophila	XenoRNA Control
F04274 (Nasal Wash)	27.17	27.16	-	30.87	-	-	-	-	-	-	31.88	-	-	-	-	-	-	-	-	-	-	-	-	31.13
	27.16	27.22	-	30.33	-	-	-	-	-	-	31.22	-	-	-	-	-	-	-	-	-	-	-	-	30.71
F40366 (Nasal Wash)	27.01	24.12	-	28.14	-	-	-	-	-	-	32.12	-	-	-	-	-	-	-	-	-	-	-	-	30.77
	27.08	24.11	-	28.10	-	-	-	-	-	-	31.06	-	-	-	-	-	-	-	-	-	-	-	-	30.28
F43541 (Nasal Wash)	27.40	25.07	-	29.66	-	-	-	-	-	-	30.29	-	-	-	-	-	-	-	-	-	-	-	-	29.94
	27.21	25.69	-	29.99	-	-	-	-	-	-	30.78	-	-	-	-	-	-	-	-	-	-	-	-	30.18
F43632 (Nasal Wash)	27.10	29.75	-	32.92	-	-	-	-	-	-	30.04	-	-	-	-	-	-	-	-	-	-	-	-	30.77
	27.74	29.53	-	33.00	-	-	-	-	-	-	30.16	-	-	-	-	-	-	-	-	-	-	-	-	30.08
NTC 1	27.25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	27.71	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NTC 2	27.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	27.80	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

pMD system

Sample	Internal Positive Control	Influenza A	Influenza B	SARS.1	RSV	Human Adenovirus Panel	Rnase P	Human PIV 3	Streptococcus pyogenes	Mycoplasma pneumoniae	Chlamydia pneumoniae	Bordetella pertussis 1	XenoRNA Control
F04274 (Nasal Wash)	25.82	23.35	-	-	-	-	30.55	-	-	-	-	-	24.33
	25.29		-	-	-	-	30.28	-	-	-	-	-	24.09
F40366 (Nasal Wash)	25.10	23.36	-	-	-	-	27.19	-	-	-	-	-	25.08
	25.09		-	-	-	-	27.38	-	-	-	-	-	25.02
F43541 (Nasal Wash)	25.74	22.31	-	-	-	-	26.78	-	-	-	-	-	24.16
	25.02		-	-	-	-	27.58	-	-	-	-	-	24.23
F43632 (Nasal Wash)	26.21	26.08	-	-	38.79	-	26.37	-	-	-	-	-	24.30
	25.47		-	-		-	27.09	-	-	-	-	-	24.31
NTC 1	26.10	-	-	-	-	-	-	-	-	-	-	-	
		-	-	-	-	-	-	-	-	-	-	-	-
NTC 2	25.82	-	-	-	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-	-
NTC 3	25.91	-	-	-	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-	-
NTC 4	26.20	-	-	-	-	-	-	-	-	-	-	-	-
		-	-	-	-	-	-	-	-	-	-	-	-

25 YEARS ADVANCING SCIENCE

24 Nasal Wash Samples run on SGURv1.0 TLDA
Florida Department of Health

Applied Biosystems

	Internal Positive Control	Influenza A	Influenza A-H1	Influenza A-H3	Influenza A-H5a	Influenza A-H5b	Influenza B	SARS.1	RSV	Human Adenovirus Panel	Rnase P	Human Adenovirus 4	Human PIV 1	Human PIV 2	Human PIV 3	Human Metapnuemovirus	Streptococcus pneumoniae	Streptococcus pyogenes	Mycoplasma pneumoniae	Chlamydia pneumoniae	Bordetella pertussis 1	Bortetella pertussis 2	Legionella pneumophila	XenoRNA Control
(Nasal Wash)	27.04	26.85	-	31.53	-	-	-	-	-	-	31.19	-	-	-	-	-	-	-	-	-	-	-	-	30.16
	27.36	26.88	-	30.56	-	-	-	-	-	-	31.42	-	-	-	-	-	-	-	-	-	-	-	-	29.95
(Nasal Wash)	27.16	35.48	-	37.93	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	29.77
	27.67	35.13	-	-	-	-	-	-	-	-	33.97	-	-	-	-	-	-	-	-	-	-	-	-	29.81
(Nasal Wash)	27.45	31.75	-	37.78	-	-	-	-	-	-	34.80	-	-	-	-	-	-	-	-	-	-	-	-	29.95
	27.46	31.95	-	36.49	-	-	-	-	-	-	38.46	-	-	-	-	-	-	-	-	-	-	15.92	-	29.03
(Nasal Wash)	27.36	30.13	-	30.13	-	-	-	-	-	-	30.45	-	-	-	-	-	-	-	-	34.82	-	-	-	29.82
	27.39	30.74	-	30.74	-	-	-	-	-	-	31.66	-	-	-	-	-	-	-	-	33.69	-	-	-	29.06
(Nasal Wash)	27.25	33.56	-	-	-	-	-	-	-	-	33.94	-	-	-	-	-	-	-	-	-	-	-	-	29.98
	27.39	35.82	-	-	-	-	-	-	-	-	34.95	-	-	-	-	-	-	-	-	-	-	-	-	30.11
(Nasal Wash)	27.07	32.39	-	35.98	-	-	-	-	-	-	32.86	-	-	-	-	-	-	-	-	-	-	-	-	30.13
	27.11	32.46	-	36.77	-	-	-	-	-	-	32.93	-	-	-	-	-	-	-	-	-	-	-	-	29.64
(Nasal Wash)	27.16	28.87	-	31.88	-	-	-	-	-	-	30.55	-	-	-	-	-	-	-	-	-	-	-	-	28.94
	27.14	28.42	-	31.83	-	-	-	-	-	-	30.99	-	-	-	-	-	28.46	-	-	-	-	-	-	29.21
(Nasal Wash)	27.24	26.65	-	31.44	-	-	-	-	-	-	29.94	-	-	-	-	-	-	-	-	-	-	-	-	30.17
	27.23	27.01	-	31.01	-	-	-	-	-	-	30.15	-	-	-	-	-	-	-	-	-	-	-	-	29.79
(Nasal Wash)	27.20	-	-	-	-	-	-	-	-	-	32.72	-	-	-	-	-	-	-	-	-	33.46	-	-	30.31
	27.09	-	-	-	-	-	-	-	-	-	34.09	-	-	-	-	-	-	-	-	-	-	-	-	30.58
(Nasal Wash)	27.22	24.50	-	28.98	-	-	-	-	-	-	31.21	-	-	-	-	-	-	-	-	-	-	-	-	30.56
	26.85	24.54	-	28.09	-	-	-	-	-	-	31.12	-	-	-	-	-	-	-	-	-	-	-	-	30.05
(Nasal Wash)	27.03	27.04	-	32.61	-	-	-	-	-	-	32.80	-	-	-	-	-	-	-	-	-	-	-	-	30.15
	27.41	27.37	-	30.75	-	-	-	-	-	-	33.98	-	-	-	-	-	-	-	-	-	-	-	-	30.20
(Nasal Wash)	27.40	30.60	-	34.70	-	-	-	-	-	-	33.91	-	-	-	-	-	-	-	-	-	-	-	-	29.75
	26.97	31.39	-	36.28	-	-	-	-	-	-	32.08	-	-	-	-	-	-	-	-	-	-	-	-	29.65
NTC 1	27.05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	27.31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NTC 2	27.19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	27.51	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

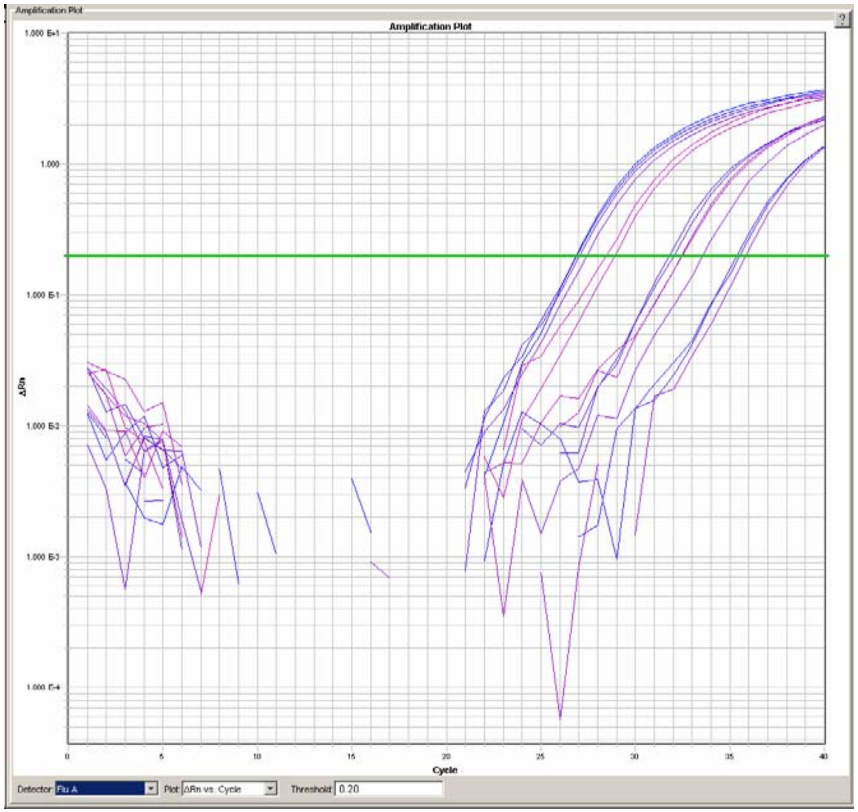
Samples 1-12

25 YEARS ADVANCING SCIENCE

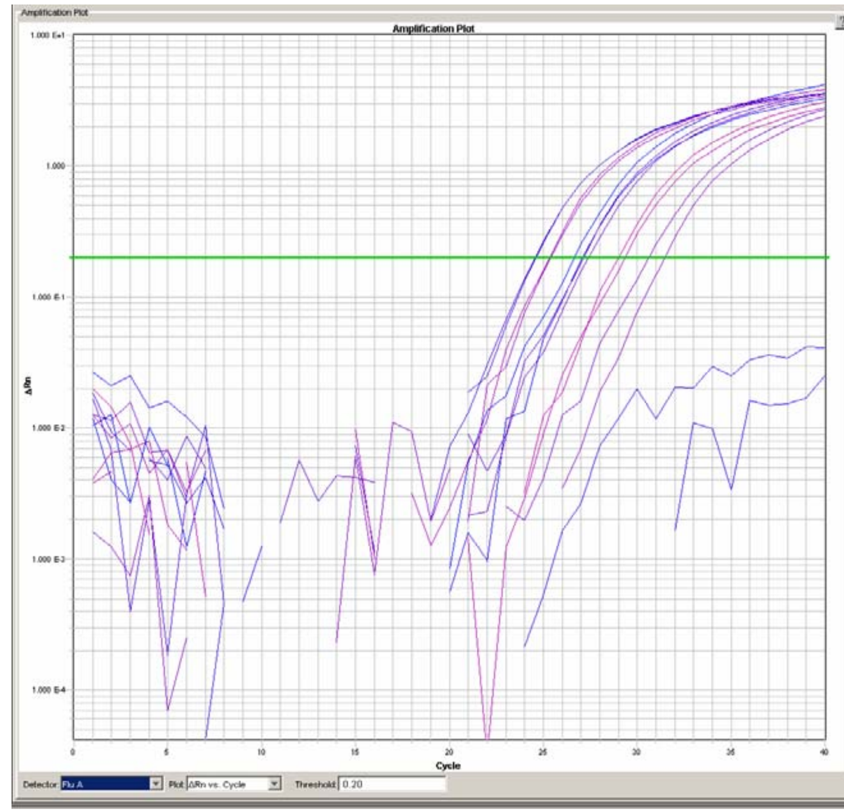
	Internal Positive Control	Influenza A	Influenza A-H1	Influenza A-H3	Influenza A-H5a	Influenza A-H5b	Influenza B	SARS.1	RSV	Human Adenovirus Panel	Rnase P	Human Adenovirus 4	Human PIV 1	Human PIV 2	Human PIV 3	Human Metapneumovirus	Streptococcus pneumoniae	Streptococcus pyogenes	Mycoplasma pneumoniae	Chlamydia pneumoniae	Bordetella pertussis 1	Bortetella pertussis 2	Legionella pneumophila	XenoRNA Control
(Nasal	27.21	25.32	-	29.34	-	-	-	-	-	-	30.53	-	-	-	-	-	-	-	-	-	-	-	-	30.01
Wash)	27.40	25.22	-	29.29	-	-	-	-	-	-	30.95	-	-	-	-	-	-	-	-	-	-	-	-	29.76
(Nasal	27.06	29.27	-	32.54	-	-	-	-	-	-	30.12	-	-	-	-	-	-	-	-	-	-	-	-	29.99
Wash)	27.02	28.98	-	33.19	-	-	-	-	-	-	29.90	-	-	-	-	-	-	-	-	-	-	-	-	29.94
(Nasal	27.70	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30.95
Wash)	27.45	-	-	19.73	-	-	-	-	-	-	34.23	-	-	-	-	-	-	-	-	-	-	-	-	30.60
(Nasal	27.14	27.47	-	30.89	-	-	-	-	-	-	30.04	-	-	-	-	-	-	-	-	-	-	-	-	31.61
Wash)	27.28	27.47	-	31.40	-	-	-	-	-	-	30.52	-	-	-	-	-	-	-	-	-	-	-	-	31.08
(Nasal	27.72	33.82	-	-	-	-	-	-	-	-	32.50	-	-	-	-	-	-	-	-	-	-	-	-	31.12
Wash)	27.56	33.90	-	36.78	-	-	-	-	-	-	32.00	-	-	-	-	-	-	-	-	-	-	-	-	30.71
(Nasal	27.15	33.80	-	37.06	-	-	-	-	-	-	32.76	-	-	-	-	-	-	-	-	-	-	-	-	30.19
Wash)	27.59	35.57	-	36.45	-	-	-	-	-	-	33.19	-	-	-	-	-	-	-	-	-	-	-	-	30.48
(Nasal	27.60	29.20	-	33.76	-	-	-	-	-	-	30.67	-	-	-	-	-	-	-	-	-	-	-	-	31.28
Wash)	27.51	29.07	-	33.35	-	-	-	-	-	-	30.04	-	-	-	-	-	-	-	-	-	-	-	-	31.01
(Nasal	27.52	28.60	-	32.57	-	-	-	-	-	-	30.09	-	-	-	-	-	-	-	-	-	-	-	-	31.37
Wash)	27.11	28.29	-	34.57	-	-	-	-	-	-	30.25	-	-	-	-	-	-	-	-	-	-	-	-	30.79
(Nasal	27.40	34.36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30.23
Wash)	27.76	36.52	-	36.22	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	30.75
(Nasal	26.99	24.95	-	28.94	-	-	-	-	-	-	30.75	-	-	-	-	-	-	-	-	-	-	-	-	33.73
Wash)	27.25	25.75	-	30.81	-	-	-	-	-	-	30.40	-	-	-	-	-	-	-	-	-	-	-	-	32.89
(Nasal	27.14	27.32	-	31.17	-	-	-	-	-	-	33.58	-	-	-	-	-	-	-	-	-	-	-	-	30.15
Wash)	27.78	27.52	-	31.15	-	-	-	-	-	-	32.59	-	-	-	-	-	-	-	-	37.22	-	-	-	30.28
(Nasal	27.10	30.86	-	34.94	-	-	-	-	-	-	32.78	-	-	-	-	-	-	-	-	-	-	-	-	30.26
Wash)	27.02	31.92	-	34.88	-	-	-	-	-	-	30.94	-	-	-	-	-	-	-	-	-	-	-	-	29.78
	27.29	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NTC 3	27.49	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	27.18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NTC 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	34.75	-	-	-	-	-	-	-

Samples 13-24

24 Nasal Wash Samples run on SGURv1.0 TLDA Florida Department of Health

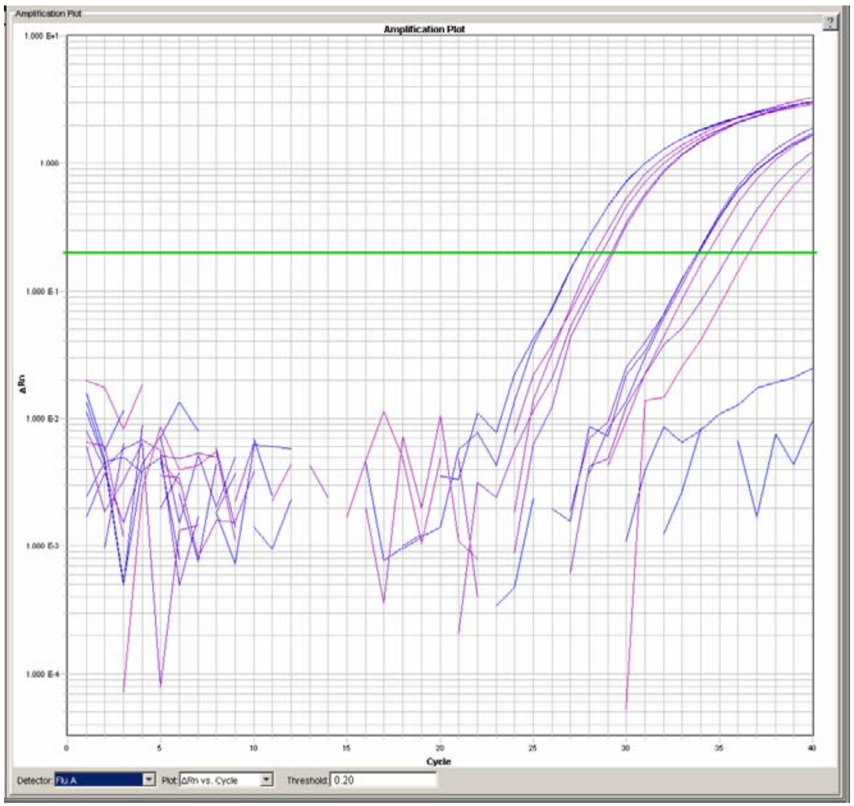


Samples 1-7

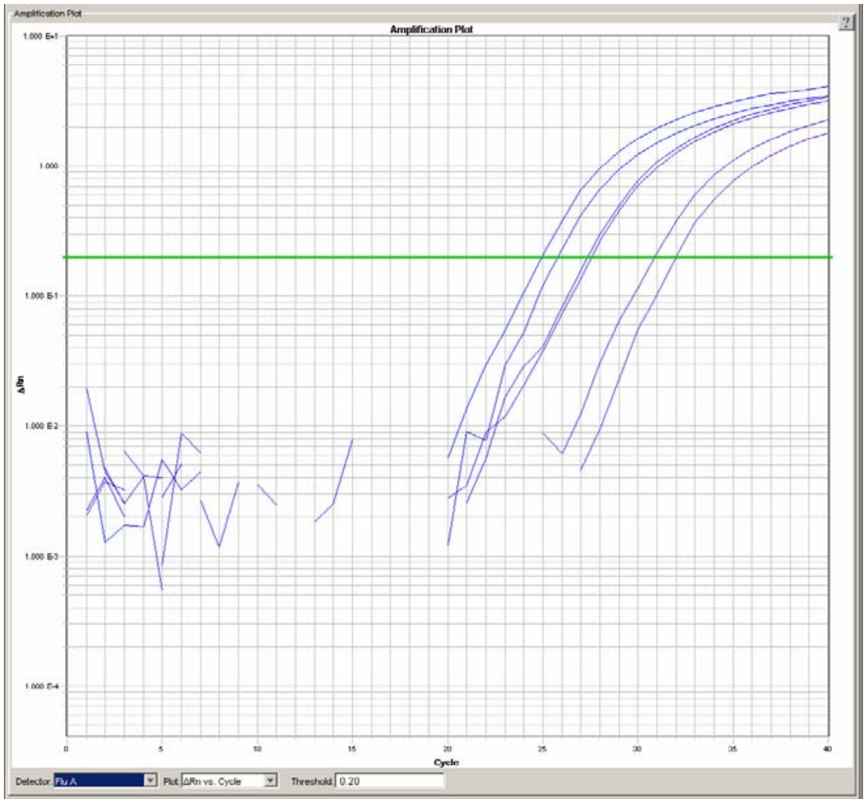


Samples 8-14

24 Nasal Wash Samples run on SGURv1.0 TLDA
Florida Department of Health

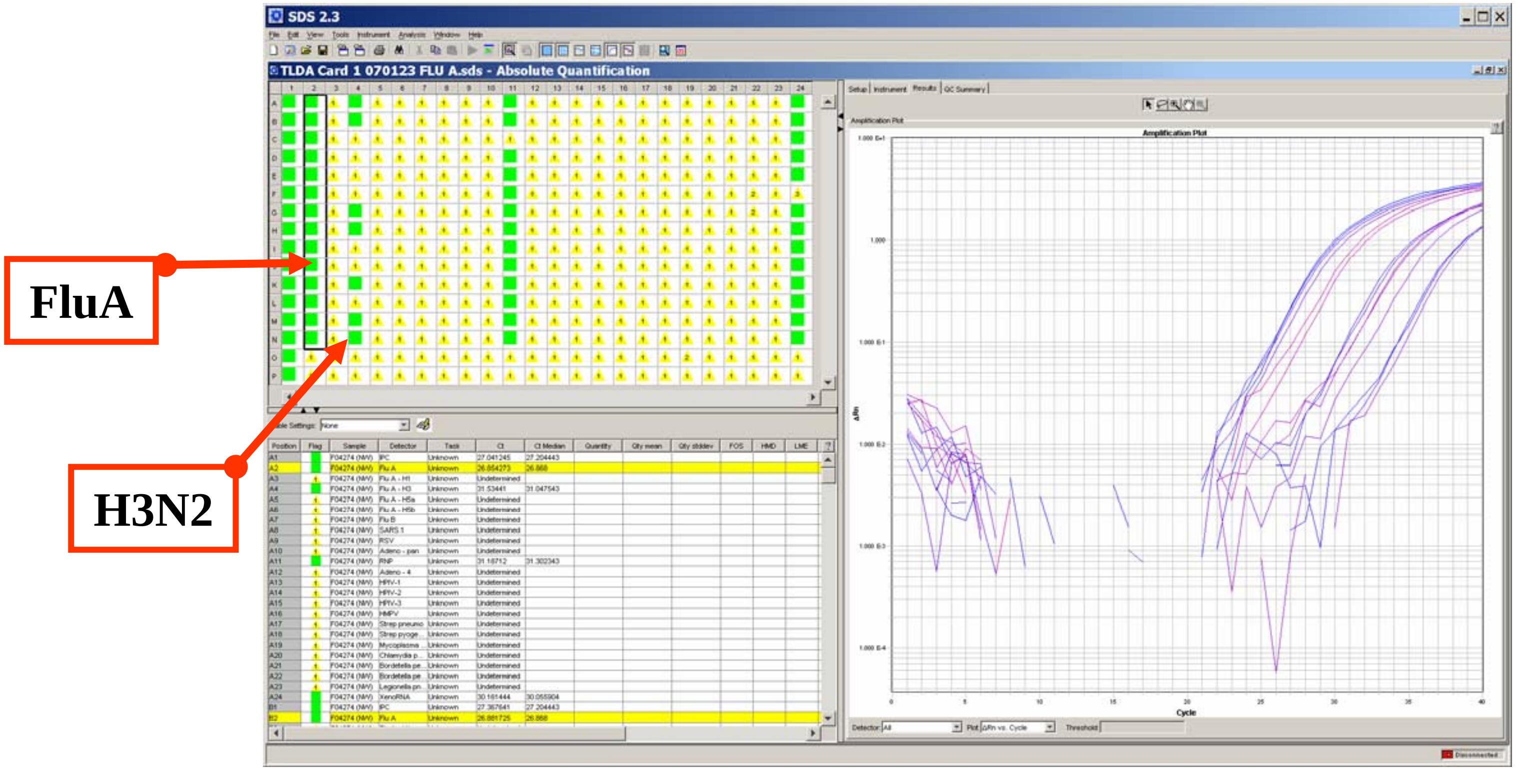


Samples 15-21



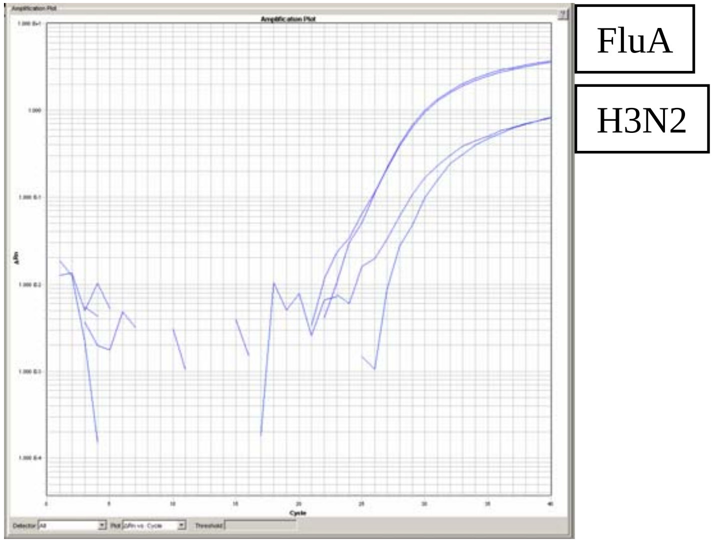
Samples 22-24

Flu A Details

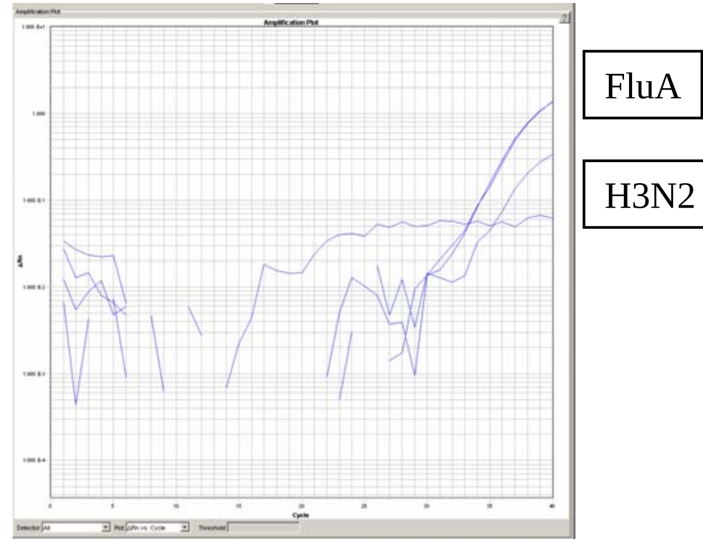


- FluA assay is positive for all 7 samples, negative for NTC
- FluA-H3N2 assay positive for 4/7; weakly positive for the remainder
- Signal from RNAseP assay varies – may indicate variation in total nucleic acid extract from each nasal wash

Flu A Details



Strong response



Weak response

- Performance of H3N2 assay significantly lags that of the FluA assay
  - Ideally, Ct values should be identical (FluA = H3N2)
- This is especially important on the lower range of target concentration
  - Subtyping unreliable on lower end
- Redesign of the H3N2 assay may correct this problem

IDIS/TLDA vs. pMD

	Influenza A		
	AB-TLDA	FLDOH-TLDA	pMD
F04274 (Nasal Wash)	27.19	26.87	23.35
F40366 (Nasal Wash)	24.12	24.52	23.36
F43541 (Nasal Wash)	25.38	25.27	22.31
F43632 (Nasal Wash)	29.64	29.13	26.08

Data on 56 blind samples

- Data from 56 “blind” culture positive samples
  - TLDA data from AB
  - TLDA data from DoH
  - pMD data (12 blind samples)

Experimental Outline

➢ Received 56 characterized samples from Lackland Air Force Base in the form of Nasal Wash and expressed Throat Swabs

- Results of characterization were unknown to AB
- However, based on previous conversations, presence of *Streptococcus pyogenes*, Human Adenovirus, and Influenza were expected

➢ 150ul of each sample was processed through the IDIS Purification Protocol at AB.

- Half of the resulting purified nucleic acid was shipped to Florida Department of Health Lab in Miami and the other half remained at AB

➢ Nucleic Acid samples were analyzed on the SGURv1.0 TLDA cards at both sites in parallel

➢ 12 Nasal Wash/Throat Swab samples were processed through the pMD Breadboard Sample Preparations System and analyzed on a pMD Card

Detection of Pathogens Across 3 Different Protocols

	EOS PIN NUMBER	SAMPLE TYPE	SAMPLE DATE	CULTURE RESULTS	IDIS/TLDA (AB) RESULTS*	IDIS/TLDA (FLDOH) RESULTS*	pMD (AB) RESULTS	
1	42579	NW	08-Sep-03	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*		
2	106422	NW	26-Aug-03	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*		
3	135870	NW	30-May-06	*Adeno*	*Adeno*	*Adeno*	*Adeno*	✓
4	207319	NW	19-Apr-06	*Adeno*	*Adeno*			
5	214962	NW	05/25/05	*B. Strep*	*Strep pyogenes*	*Strep pyogenes*	*pyogenes, Chlaymydia pneumo*	✓
6	214962	TS	05/25/05	*B. Strep*	*Strep pyogenes*	*Strep pyogenes*	*Strep pyogenes*	✓
7	225901	NW	02/15/06	*B. Strep*				
8	225901	TS	02/15/06	*B. Strep*	*Strep pyogenes*	*Strep pyogenes*		
9	237876	NW	18-Oct-04	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*		
10	239078	NW	27-Feb-03	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*		
11	300563	NW	22-Oct-04	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*		
12	PN001	NW	22-Jun-05	*Normal Pool*	*Strep pneumo*			✓
13	302818	NW	26-Apr-05	*Adeno*	*Adeno*	*Adeno*		
14	361614	NW	06-Oct-04	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*	*Adeno*	✓
15	389304	NW	04-Oct-04	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*		
16	459648	NW	24-Nov-04	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*		
17	518251	NW	27-Feb-03	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*		
18	551184	NW	03/08/05	*Flu B*	*Flu B*	*Flu B*		

✓ = pMD / culture agreement

Samples analyzed on pMD (highlighted rows: 3, 5, 6, 12, 14)

Detection of Pathogens Across 3 Different Protocols

Applied Biosystems

	EOS PIN NUMBER	SAMPLE TYPE	SAMPLE DATE	*CULTURE RESULTS*	*IDIS/TLDA (AB) RESULTS**	*IDIS/TLDA (FLDOH) RESULTS**	*pMD (AB) RESULTS*
19	608634	NW	05-Sep-03	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*	
20	626278	NW	27-Sep-04	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*	*Adeno*
21	675208	NW	05-Oct-04	*Adeno*	*Adeno, Adeno 4*	*Adeno, Adeno 4*	
22	703667	NW	05/11/06	*B. Strep*			
23	PN001	NW	22-Jun-05	*Normal Pool*	*Strep pneumo*	*Strep pneumo*	
24	703667	TS	05/11/06	*B. Strep*			
25	740886	NW	02/13/06	*B. Strep*	*Flu A, Flu A-H3*	*Flu A, Flu A-H3*	*Flu A*
26	740886	TS	02/13/06	*B. Strep*			
27	823112	NW	03/02/06	*B. Strep*			
28	823112	TS	03/02/06	*B. Strep*	*Strep pyogenes*		
29	F38123	NW	02/17/06	*B. Strep*	*Strep pyogenes*	*Strep pyogenes*	
30	F38123	TS	02/17/06	*B. Strep*	*Strep pyogenes*	*Strep pyogenes*	*Strep pyogenes*
31	F43172	NW	12/13/05	*B. Strep*			
32	F43172	TS	12/13/05	*B. Strep*			
33	F70665	NW	12/12/05	*B. Strep*			
34	F70665	TS	12/12/05	*B. Strep*			
35	F75134	NW	05/01/06	*B. Strep*	*Strep pyogenes*	*Strep pyogenes*	
36	PN001	NW	22-Jun-05	*Normal Pool*			
37	F75134	TS	05/01/06	*B. Strep*	*Strep pyogenes*	*Strep pyogenes*	

Samples analyzed on pMD

X – AB Strep culture results for 74086 **TS** only show normal flora

X – AB Strep culture results for F43172 **TS** only show normal flora

Detection of Pathogens Across 3 Different Protocols

Applied Biosystems

	EOS PIN NUMBER	SAMPLE TYPE	SAMPLE DATE	CULTURE RESULTS	IDIS/TLDA (AB) RESULTS*	IDIS/TLDA (FLDOH) RESULTS*	pMD (AB) RESULTS
38	F80062	TS	12/07/05	B. Strep	Strep pyogenes	Strep pyogenes	
39	F86289	NW	11/03/05	B. Strep	HMPV	HMPV	
40	F86289	TS	11/03/05	B. Strep	Strep pyogenes	Strep pyogenes	
41	F90223	TS	05/04/06	B. Strep	Strep pyogenes	Strep pyogenes	
42	FE2702	NW	04/05/06	Flu B	Flu B	Flu B	
43	FE3129	NW	03/07/05	Flu B	Flu B	Flu B	
44	FE3497	NW	03/08/06	Flu B	Flu B	Flu B	Flu B
45	FE4657	NW	05/22/06	Flu B	(IPC and Xeno controls failed)	Flu B	Flu B
46	FE5809	NW	01/29/05	Flu B	Flu B	Flu B	
47	PN001	NW	22-Jun-05	Normal Pool	Strep pneumo	Strep pneumo	
48	FE5982	NW	04/24/06	Flu B	Flu B	Flu B	
49	FE6105	NW	05/11/06	Flu B			
50	FE6116	NW	03/02/05	Flu B	Flu B	Flu B	
51	FE6161	NW	03/01/05	Flu B	Flu B	Flu B	
52	FE6441	NW	01/18/05	Flu B	Flu B, Strep pneumo	Flu B, Strep pneumo	
53	FE6923	NW	04/10/06	Flu B	Flu B	Flu B	Flu B
54	FE7112	NW	04/19/06	Flu B	Flu B	Flu B	
55	FE7224	NW	04/26/06	Flu B	Flu B	Flu B	
56	PN001	NW	22-Jun-05	Normal Pool	Strep pneumo	Strep pneumo	

Samples analyzed on pMD

25 YEARS ADVANCING SCIENCE

IDIS/TLDA
(AB and Florida Department of Health)

Applied Biosystems

Applied Biosystems

Sample ID	Internal Positive Control	Influenza A	Influenza A-H3	Influenza B	Human Adenovirus Panel	Rnase P	Human Adenovirus 4	Human Metapnuemovirus	Streptococcus pneumoniae	Streptococcus pyogenes	Mycoplasma pneumoniae	XenoRNA Control
42579	27.54	-	-	-	31.09	31.83	25.85	-	-	-	-	30.92
	27.87	-	-	-	30.23	32.60	26.16	-	-	-	-	31.22
106422	27.51	-	-	-	32.28	28.43	27.93	-	-	-	-	32.75
	27.49	-	-	-	32.11	28.83	27.88	-	-	-	-	31.84
135870	27.62	-	-	-	38.16	28.44	-	-	-	-	-	32.93
	27.78	-	-	-	36.91	28.82	-	-	-	-	-	31.85
207319	27.70	-	-	-	35.22	30.61	-	-	-	-	-	31.12
	27.41	-	-	-	34.46	30.66	-	-	-	-	-	31.00
214962	27.44	-	-	-	-	27.18	-	-	-	37.21	-	32.50
	27.67	-	-	-	-	26.94	-	-	-	35.55	-	31.85
214962 (TS)	27.58	-	-	-	-	27.29	-	-	-	30.48	-	31.37
	28.27	-	-	-	-	27.20	-	-	-	30.26	35.97	31.72
225901	27.60	-	-	-	-	28.96	-	-	-	-	-	31.01
	27.85	-	-	-	-	29.61	-	-	-	-	36.87	31.72
225901 (TS)	26.97	-	-	-	-	30.01	-	-	-	31.08	-	32.46
	27.31	-	-	-	-	29.41	-	-	-	31.31	-	33.48
237876	27.36	-	-	-	25.85	29.46	21.66	-	-	-	-	32.19
	27.37	-	-	-	25.86	29.70	21.90	-	-	-	-	31.47
239078	27.14	-	-	-	27.84	29.41	23.89	-	-	-	-	31.43
	27.45	-	-	-	27.80	30.13	23.95	-	-	-	-	31.54
300563	27.67	-	-	-	27.49	25.97	22.41	-	-	-	-	35.09
	27.78	-	-	-	27.08	26.13	22.22	-	-	-	-	33.67
PN001 (TS)	27.30	-	-	-	-	28.97	-	-	34.96	-	-	31.47
	27.73	-	-	-	-	29.28	-	-	34.02	-	-	31.24
302818	27.36	-	-	-	32.51	29.37	-	-	-	-	-	31.50
	27.37	-	-	-	32.44	30.03	-	-	39.97	-	-	32.41
361614	27.63	-	-	-	31.22	30.39	25.96	-	-	-	-	33.01
	27.97	-	-	-	31.37	30.30	26.23	-	-	-	-	33.92
389304	27.58	-	-	-	22.76	27.77	19.12	-	-	-	-	-
	27.42	-	-	-	23.14	27.63	18.87	-	-	-	-	33.42
459648	27.17	-	-	-	26.49	30.03	22.71	-	-	-	-	38.35
	27.16	-	-	-	26.38	29.57	22.92	-	-	-	-	32.44
518251	27.24	-	-	-	24.24	24.94	21.44	-	-	-	-	30.51
	27.43	-	-	-	24.42	24.95	21.70	-	-	-	-	30.57
551184	27.27	-	-	28.53	-	27.74	-	-	-	-	-	30.38
	27.29	-	-	28.28	-	27.64	-	-	-	-	-	30.33

FL Dept. of Health

Sample ID	Internal Positive Control	Influenza A	Influenza A-H3	Influenza B	Human Adenovirus Panel	Rnase P	Human Adenovirus 4	Human Metapnuemovirus	Streptococcus pneumoniae	Streptococcus pyogenes	Mycoplasma pneumoniae	XenoRNA Control
42579	27.85	-	-	-	30.61	32.39	26.26	-	-	-	-	30.21
	27.95	-	-	-	30.51	36.35	26.82	-	-	-	-	30.61
106422	27.80	-	-	-	33.07	29.66	29.23	-	-	-	-	30.43
	27.65	-	-	-	33.08	29.58	29.29	-	-	-	-	30.53
135870	27.56	-	-	-	36.99	29.07	-	-	-	-	-	-
	28.09	-	-	-	37.99	29.52	-	-	-	-	-	31.61
207319	27.75	-	-	-	-	31.12	-	-	-	-	-	30.43
	27.49	-	-	-	-	31.27	-	-	-	-	-	29.90
214962	27.70	-	-	-	-	27.78	-	-	-	34.29	-	31.27
	27.50	-	-	-	-	27.67	-	-	-	33.72	-	31.13
214962 (TS)	27.39	-	-	-	-	27.49	-	-	-	30.40	-	31.51
	27.20	-	-	-	-	27.76	-	-	-	30.54	-	30.68
225901	26.59	-	-	-	-	30.32	-	-	-	-	-	30.54
	27.49	-	-	-	-	29.40	-	-	-	-	-	30.28
225901 (TS)	27.60	-	-	-	-	30.79	-	-	-	32.13	-	30.59
	28.11	-	-	-	-	30.45	-	-	-	31.59	-	30.68
237876	27.71	-	-	-	26.57	30.88	22.66	-	-	-	-	30.75
	27.59	-	-	-	26.44	30.49	22.70	-	-	-	-	30.90
239078	27.66	-	-	-	28.44	30.70	24.59	-	-	-	-	30.49
	28.03	-	-	-	28.21	30.26	24.55	-	-	-	-	30.68
300563	27.57	-	-	-	26.97	27.01	23.13	-	-	-	-	31.32
	27.81	-	-	-	26.93	26.95	22.97	-	-	-	-	31.51
PN001 (TS)	27.48	-	-	-	-	29.89	-	-	35.45	-	-	30.62
	27.52	-	-	-	-	30.54	-	-	-	-	-	30.81
302818	27.53	-	-	-	32.71	30.36	-	-	-	-	-	30.79
	27.43	-	-	-	32.22	30.53	-	-	-	-	-	30.76
361614	27.53	-	-	-	31.06	30.61	26.88	-	-	-	-	31.16
	27.94	-	-	-	31.14	31.24	26.68	-	-	-	-	31.14
389304	27.47	-	-	-	23.35	28.38	19.28	-	-	-	-	31.14
	27.84	-	-	-	23.82	28.72	19.85	-	-	-	-	31.32
459648	27.46	-	-	-	27.14	30.17	23.84	-	-	-	-	30.80
	27.52	-	-	-	27.26	30.15	23.40	-	-	-	-	30.77
518251	27.00	-	-	-	25.31	25.94	22.31	-	-	-	-	29.60
	27.74	-	-	-	25.19	25.83	22.40	-	-	-	-	29.76
551184	27.45	-	-	27.88	-	28.67	-	-	-	-	-	29.46
	27.69	-	-	27.24	-	28.35	-	-	-	-	-	29.67

Pathogens detected matching culture test results

Other pathogens detected

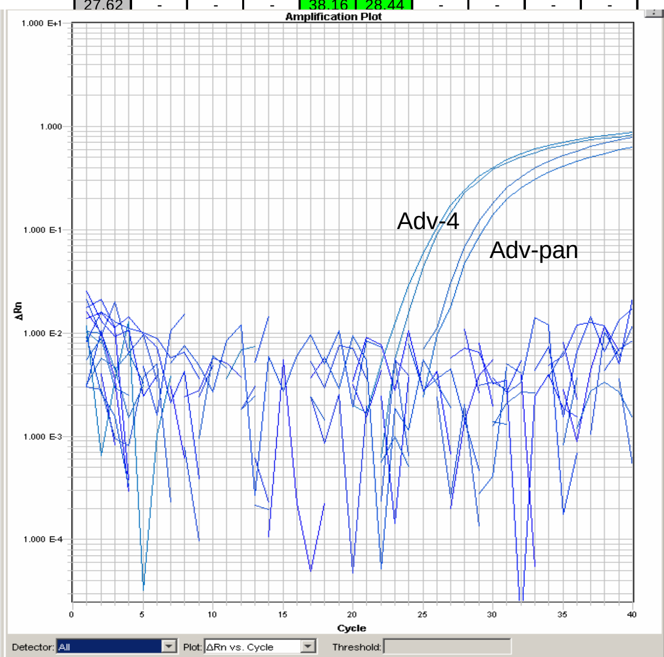
Controls

IDIS/TLDA
(AB and Florida Department of Health)

Applied Biosystems

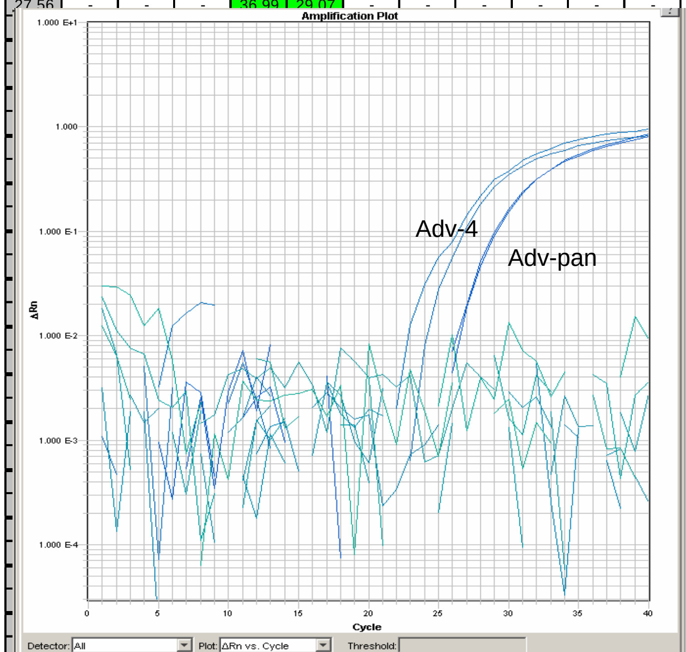
Applied Biosystems

ample ID	nternal Positive Control	nfluenza A	nfluenza A-H3	nfluenza B	uman Adenovirus Panel	Rnase P	uman Adenovirus 4	uman Metapnuemovirus	treptococcus pneumoniae	treptococcus pyogenes	ycoplasma pneumoniae	enoRNA Control
	27.54	-	-	-	31.09	31.83	25.85	-	-	-	-	30.92
42579	27.87	-	-	-	30.23	32.60	26.16	-	-	-	-	31.22
	27.51	-	-	-	32.28	28.43	27.93	-	-	-	-	32.75
106422	27.49	-	-	-	32.11	28.83	27.88	-	-	-	-	31.84
	27.62	-	-	-	38.16	28.44	-	-	-	-	-	32.93
13												31.85
												31.12
20												31.00
												32.50
21												31.85
												31.37
214											97	31.72
												31.01
22											87	31.72
												32.46
225												33.48
												32.19
23												31.47
												31.43
23												31.54
												35.09
30												33.67
												31.47
PNC												31.24
												31.50
30												32.41
												33.01
36												33.92
												-
38												33.42
												38.35
45												32.44
	27.24	-	-	-	24.24	24.94	21.44	-	-	-	-	30.51
518251	27.43	-	-	-	24.42	24.95	21.70	-	-	-	-	30.57
	27.27	-	-	28.53	-	27.74	-	-	-	-	-	30.38
551184	27.29	-	-	28.28	-	27.64	-	-	-	-	-	30.33



FL Dept. of Health

nternal Positive Control	nfluenza A	nfluenza A-H3	nfluenza B	uman Adenovirus Panel	Rnase P	uman Adenovirus 4	uman Metapnuemovirus	treptococcus pneumoniae	treptococcus pyogenes	ycoplasma pneumoniae	enoRNA Control
27.85	-	-	-	30.61	32.39	26.26	-	-	-	-	30.21
27.95	-	-	-	30.51	36.35	26.82	-	-	-	-	30.61
27.80	-	-	-	33.07	29.66	29.23	-	-	-	-	30.43
27.65	-	-	-	33.08	29.58	29.29	-	-	-	-	30.53
27.56	-	-	-	36.99	29.07	-	-	-	-	-	-
27.00	-	-	-	25.31	25.94	22.31	-	-	-	-	29.60
27.74	-	-	-	25.19	25.83	22.40	-	-	-	-	29.76
27.45	-	-	27.88	-	28.67	-	-	-	-	-	29.46
27.69	-	-	27.24	-	28.35	-	-	-	-	-	29.67



Pathogens detected matching culture test results

Other pathogens detected

Controls

25 YEARS ADVANCING SCIENCE

IDIS/TLDA
(AB and Florida Department of Health)

Applied Biosystems

Applied Biosystems

Sample ID	Internal Positive Control	Influenza A	Influenza A-H3	Influenza B	Human Adenovirus Panel	Rnase P	Human Adenovirus 4	Human Metapnuemovirus	Streptococcus pneumoniae	Streptococcus pyogenes	Mycoplasma pneumoniae	XenoRNA Control
608634	26.94	-	-	-	26.80	25.89	23.78	-	-	-	-	31.00
	27.48	-	-	-	26.63	25.85	23.71	-	-	-	-	30.56
626278	27.74	-	-	-	23.97	28.36	20.75	-	-	-	-	30.40
	27.84	-	-	-	23.92	28.80	20.65	-	-	-	-	30.87
675208	27.32	-	-	-	27.73	28.70	24.45	-	-	-	-	30.66
	27.30	-	-	-	27.52	28.56	24.32	-	-	-	-	30.90
703667	29.01	-	-	-	-	31.81	-	-	-	-	-	34.70
	29.01	-	-	-	-	31.64	-	-	-	-	-	35.40
PN001 (TS)	27.75	-	-	-	-	29.02	-	-	34.16	-	-	31.15
	28.03	-	-	-	-	29.01	-	-	34.24	-	-	30.73
703667 (TS)	27.65	-	-	-	-	27.30	-	-	-	-	-	30.98
	28.10	-	-	-	-	27.29	-	-	-	-	-	31.47
740886	27.75	26.41	35.62	-	-	26.56	-	-	-	-	-	30.41
	27.97	26.42	35.10	-	-	26.46	-	-	-	-	-	30.48
740886 (TS)	27.60	-	-	-	-	30.41	-	-	-	-	-	31.30
	28.16	35.85	-	-	-	30.37	-	-	-	-	-	30.86
823112	28.06	-	-	-	-	34.10	-	-	-	35.37	-	31.37
	28.28	-	-	-	-	34.94	-	-	-	-	-	31.57
823112 (TS)	28.12	-	-	-	-	30.71	-	-	-	34.69	-	31.54
	28.08	-	-	-	-	31.09	-	-	-	33.15	-	31.98
F38123	27.56	-	-	-	-	27.36	-	-	-	28.17	-	31.12
	27.54	-	-	-	-	27.32	-	-	-	28.09	-	31.14
F38123 (TS)	27.64	-	-	-	-	25.43	-	-	-	25.06	-	33.00
	27.78	-	-	-	-	25.71	-	-	-	25.12	-	33.14
F43172	27.52	-	-	-	-	29.94	-	-	-	-	-	35.35
	27.53	-	-	-	-	30.03	-	-	-	-	-	33.90
F43172 (TS)	27.74	-	-	-	-	30.51	-	-	-	-	-	31.66
	27.86	-	-	-	-	30.36	-	-	-	-	-	32.09
F70665	27.39	-	-	-	-	27.12	-	-	-	-	-	30.87
	27.47	-	-	-	-	27.22	-	-	-	-	-	30.56
F70665 (TS)	27.76	-	-	-	-	27.07	-	-	-	-	-	31.48
	27.62	-	-	-	-	27.24	-	-	-	-	-	31.29
F75134	27.97	-	-	-	-	29.95	-	-	-	27.40	-	31.82
	28.07	-	-	-	-	30.51	-	-	-	27.50	-	32.10
PN001 (TS)	27.39	-	-	-	-	29.00	-	-	-	-	-	31.86
	27.60	-	-	-	-	29.35	-	-	-	-	-	32.28

FL Dept. of Health

Sample ID	Internal Positive Control	Influenza A	Influenza A-H3	Influenza B	Human Adenovirus Panel	Rnase P	Human Adenovirus 4	Human Metapnuemovirus	Streptococcus pneumoniae	Streptococcus pyogenes	Mycoplasma pneumoniae	XenoRNA Control
608634	27.54	-	-	-	27.20	26.12	24.10	-	-	-	-	30.45
	27.53	-	-	-	26.84	26.01	23.96	-	-	-	-	29.45
626278	27.66	-	-	-	23.90	28.51	20.86	-	-	-	-	29.94
	27.61	-	-	-	23.82	29.00	20.83	-	-	-	-	29.71
675208	27.19	-	-	-	28.13	28.67	25.11	-	-	-	-	30.15
	27.29	-	-	-	28.33	28.69	25.16	-	-	-	-	29.93
703667	27.91	-	-	-	-	31.96	-	-	-	-	-	31.55
	28.34	-	-	-	-	31.68	-	-	-	-	-	31.82
PN001 (TS)	27.24	-	-	-	-	29.39	-	-	33.83	-	-	29.69
	27.64	-	-	-	-	29.16	-	-	35.33	-	-	29.77
703667 (TS)	27.32	-	-	-	-	27.69	-	-	-	-	-	29.62
	27.72	-	-	-	-	27.74	-	-	-	-	-	29.60
740886	27.16	25.53	31.56	-	-	26.33	-	-	-	-	-	28.52
	27.41	25.76	32.38	-	-	26.40	-	-	-	-	-	29.43
740886 (TS)	27.22	36.05	-	-	-	29.70	-	-	-	-	-	30.26
	27.08	-	-	-	-	30.18	-	-	-	-	-	30.37
823112	27.11	-	-	-	-	32.63	-	-	-	34.46	-	29.46
	27.04	-	-	-	-	-	-	-	-	-	-	29.62
823112 (TS)	26.94	-	-	-	-	30.46	-	-	-	33.17	-	30.05
	27.22	-	-	-	-	30.81	-	-	-	-	-	30.09
F38123	27.23	-	-	-	-	27.41	-	-	-	28.51	-	30.76
	27.82	-	-	-	-	27.59	-	-	-	28.54	-	30.52
F38123 (TS)	27.34	-	-	-	-	25.94	-	-	-	25.25	-	31.11
	27.83	-	-	-	-	25.92	-	-	-	25.19	-	31.90
F43172	27.53	-	-	-	-	30.23	-	-	-	-	-	31.38
	28.26	-	-	-	-	30.24	-	-	-	-	-	31.02
F43172 (TS)	27.47	-	-	-	-	30.60	-	-	-	-	-	30.74
	27.77	-	-	-	-	30.43	-	-	-	-	-	30.56
F70665	27.47	-	-	-	-	27.56	-	-	-	-	-	30.57
	27.42	-	-	-	-	27.51	-	-	-	-	-	30.27
F70665 (TS)	27.51	-	-	-	-	27.44	-	-	-	-	-	30.97
	27.33	-	-	-	-	27.41	-	-	-	-	-	31.04
F75134	27.22	-	-	-	-	30.22	-	-	-	27.61	-	30.88
	27.82	-	-	-	-	29.86	-	-	-	27.42	-	30.93
PN001 (TS)	27.39	-	-	-	-	29.49	-	-	-	-	-	32.36
	27.58	-	-	-	-	29.54	-	-	35.31	-	-	32.87

Pathogens detected matching culture test results

Other pathogens detected

Controls

IDIS/TLDA
(AB and Florida Department of Health)

Applied Biosystems

Applied Biosystems

Sample ID	Internal Positive Control	Influenza A	Influenza A-H3	Influenza B	Human Adenovirus Panel	Rnase P	Human Adenovirus 4	Human Metapnuemovirus	Streptococcus pneumoniae	Streptococcus pyogenes	Mycoplasma pneumoniae	XenoRNA Control
F75134 (TS)	27.50	-	-	-	-	29.33	-	-	-	31.37	-	35.50
	27.64	-	-	-	-	29.86	-	-	-	31.89	-	34.98
F80062 (TS)	27.98	-	-	-	-	26.16	-	-	-	27.36	-	34.66
	27.82	-	-	-	-	26.28	-	-	-	28.40	-	34.77
F86289	27.48	-	-	-	-	27.55	-	37.86	-	37.00	-	34.49
	27.49	-	-	-	-	27.53	-	37.34	-	-	-	33.86
F86289 (TS)	27.43	-	-	-	-	28.37	-	-	-	30.64	-	31.40
	27.72	-	-	-	-	28.91	-	-	-	30.92	-	31.82
F90223	27.48	-	-	-	-	29.79	-	-	-	27.86	-	31.21
	27.79	-	-	-	-	29.25	-	-	-	27.70	-	31.90
FE2702	27.93	-	-	28.77	-	30.09	-	-	-	-	-	35.23
	27.86	-	-	28.96	-	29.49	-	-	-	-	-	35.71
FE3129	27.42	-	-	24.79	-	22.43	-	-	-	-	-	32.53
	27.32	-	-	25.01	-	22.43	-	-	-	-	-	33.20
FE3497	27.15	-	-	31.22	-	28.83	-	-	-	-	-	33.04
	27.29	-	-	31.61	-	29.11	-	-	-	-	-	33.11
FE4657	-	-	-	-	-	21.98	-	-	-	-	-	-
	-	-	-	-	-	21.66	-	-	-	-	-	-
FE5809	27.56	-	-	32.06	-	29.55	-	-	-	-	-	33.32
	27.40	-	-	31.52	-	29.64	-	-	-	-	-	33.35
PN001 (TS)	27.38	-	-	-	-	28.65	-	-	34.77	-	-	33.40
	27.45	-	-	-	-	28.70	-	-	36.16	-	-	33.25
FE5982	27.13	-	-	27.42	-	30.83	-	-	-	-	-	31.81
	27.52	-	-	27.46	-	30.89	-	-	-	-	-	31.57
FE6105	27.48	-	-	-	-	27.88	-	-	-	-	-	30.61
	27.50	-	-	-	-	27.43	-	-	-	-	-	30.45
FE6116	27.33	-	-	29.88	-	27.26	-	-	-	-	-	30.98
	27.38	-	-	29.57	-	27.34	-	-	-	-	-	30.71
FE6161	27.43	-	-	29.24	-	30.46	-	-	-	-	-	31.19
	27.47	-	-	29.58	-	30.00	-	-	-	-	-	30.75
FE6441	27.77	-	-	25.75	-	23.29	-	-	25.24	-	-	35.14
	28.11	-	-	25.71	-	23.41	-	-	24.97	-	-	34.70
FE6923	27.14	-	-	34.95	-	28.35	-	-	-	-	-	30.64
	27.61	-	-	36.03	-	28.02	-	-	-	-	-	30.94
FE7112	27.92	-	-	24.96	-	21.62	-	-	-	-	-	35.88
	27.65	-	-	24.77	-	21.70	-	-	-	-	-	35.61
FE7224	27.47	-	-	33.45	-	29.36	-	-	-	-	-	31.96
	27.60	-	-	32.40	-	29.00	-	-	-	-	-	31.41
PN001 (TS)	27.67	-	-	-	-	29.55	-	-	34.63	-	-	31.03
	27.27	-	-	-	-	28.89	-	-	35.75	-	-	31.71

FL Dept. of Health

Sample ID	Internal Positive Control	Influenza A	Influenza A-H3	Influenza B	Human Adenovirus Panel	Rnase P	Human Adenovirus 4	Human Metapnuemovirus	Streptococcus pneumoniae	Streptococcus pyogenes	Mycoplasma pneumoniae	XenoRNA Control
F75134 (TS)	27.25	-	-	-	-	29.30	-	-	-	31.00	-	32.31
	27.61	-	-	-	-	29.85	-	-	-	30.84	-	32.39
F80062 (TS)	27.21	-	-	-	-	26.66	-	-	-	27.45	-	32.50
	27.58	-	-	-	-	26.65	-	-	-	27.87	-	33.24
F86289	27.32	-	-	-	-	28.22	-	36.43	-	-	-	33.21
	27.73	-	-	-	-	27.99	-	34.98	-	35.71	-	31.98
F86289 (TS)	26.97	-	-	-	-	29.34	-	-	-	31.04	-	31.34
	27.51	-	-	-	-	29.22	-	38.22	-	31.01	-	32.20
F90223	27.43	-	-	-	-	29.99	-	-	-	27.99	-	32.19
	27.43	-	-	-	-	29.88	-	-	-	28.27	-	31.61
FE2702	27.31	-	-	29.64	-	29.98	-	-	-	-	-	32.36
	27.59	-	-	29.45	-	31.56	-	-	-	-	-	32.77
FE3129	27.22	-	-	23.66	-	22.84	-	-	-	-	-	30.39
	27.61	-	-	23.93	-	22.84	-	-	-	-	-	30.37
FE3497	27.07	-	-	29.32	-	28.56	-	-	-	-	-	28.91
	27.60	-	-	29.86	-	28.84	-	-	-	-	-	29.67
FE4657	28.06	-	-	23.44	-	19.62	-	-	-	-	-	38.58
	28.64	-	-	23.59	-	19.68	-	-	-	-	-	36.57
FE5809	27.41	-	-	29.87	-	29.36	-	-	-	-	-	29.93
	27.66	-	-	29.48	-	29.60	-	-	-	-	-	29.89
PN001 (TS)	27.28	-	-	-	-	28.67	-	-	32.78	-	-	30.11
	27.31	-	-	-	-	28.77	-	-	33.93	-	-	29.85
FE5982	27.29	-	-	26.31	-	30.75	-	-	-	-	-	29.55
	27.39	-	-	26.25	-	30.23	-	-	-	-	-	29.46
FE6105	27.12	-	-	34.44	-	27.75	-	-	-	-	-	29.37
	27.40	-	-	-	-	27.94	-	-	-	-	-	29.08
FE6116	27.68	-	-	28.55	-	27.77	-	-	-	-	-	29.75
	27.80	-	-	28.80	-	27.82	-	-	-	-	-	30.18
FE6161	27.51	-	-	28.30	-	30.18	-	-	-	-	-	31.84
	27.73	-	-	28.04	-	30.73	-	-	-	-	-	30.30
FE6441	27.65	-	-	24.03	-	23.52	-	-	23.28	-	-	-
	27.99	-	-	24.01	-	23.51	-	-	23.20	-	-	31.49
FE6923	27.64	-	-	35.93	-	28.41	-	-	-	-	-	30.92
	28.08	-	-	35.15	-	28.64	-	-	-	-	-	30.57
FE7112	27.69	-	-	23.25	-	21.25	-	-	-	-	-	31.47
	27.88	-	-	23.27	-	21.21	-	-	-	-	-	31.32
FE7224	27.63	-	-	31.46	-	29.22	-	-	-	-	-	30.37
	27.69	-	-	32.02	-	29.44	-	-	-	-	-	30.21
PN001 (TS)	27.71	-	-	-	-	29.01	-	-	33.09	-	-	30.00
	27.80	-	-	-	-	28.86	-	-	34.88	-	-	29.97

Pathogens detected matching culture test results

Other pathogens detected

Controls

APPLIED BIOSYSTEMS PROPRIETARY INFORMATION COVERED BY CONFIDENTIALITY AND NONDISCLOSURE BETWEEN APPLIED BIOSYSTEMS AND USAF

 AB CONFIDENTIAL

25 YEARS ADVANCING SCIENCE

IDIS/TLDA vs. pMD

	Flu B			Adeno-pan			S. pyo			Flu A		
	TLDA-AB	TLDA-FLDOH	pMD	TLDA-AB	TLDA-FLDOH	pMD	TLDA-AB	TLDA-FLDOH	pMD	TLDA-AB	TLDA-FLDOH	pMD
FE6923	35.49	35.54	29.11									
FE4657	Controls Failed	23.51	23.73									
135870				37.54	37.49	29.34						
740886										26.41	25.65	24.26
PN001 (TS)												
214962							36.38	34.01	34.41			
361614				31.29	31.10	28.57						
214962 (TS)							30.37	30.47	31.72			
FE3497	31.41	29.59	26.53									
F43172												
626278				23.94	23.86	25.06						
F38123 (TS)							25.09	25.22	26.49			

There is good correlation between what the pMD system and the IDIS / TLDA system detect in both sets of experiments

Nasal Wash/Throat Swab Study (Summary)

Applied Biosystems

The Final Tally…

- Correlation between TLDA(AB) and TLDA(FLDOH) for Tier 1 pathogens 53/56 (94.6%)
- Correlation between TLDA(AB) and culture results for Tier 1 pathogens 43/56 (76.8%)
- Correlation between TLDA (FLDOH) and culture results for Tier 1 pathogens 42/56 (75%)
- Correlation between pMD(AB) and culture results for Tier 1 pathogens (12 samples) 10/12 (83.3%)

  ** 1 of the mis-matches was same #25*

25 YEARS ADVANCING SCIENCE

Idaho Technology FilmArray Instrument Data

Mark Poritz, PhD.
mark_poritz@idahotech.com

The following pages show the data from FilmArray instrument runs in which either the yeast Saccharomyces cerevisiae (Sc) or Schizosaccharomyces pombe (Sp) were injected into a FilmArray pouch containing lyophilized reagents. The pouch was put into the FilmArray instrument and run in a one-hour protocol. Each page shows the amplification curves and the amplicon melt profiles for a single run.

PCR arrays were hand spotted in the runs before April and a microarrayer (Nanoplotter, GeSiM, Germany) was used after that date.

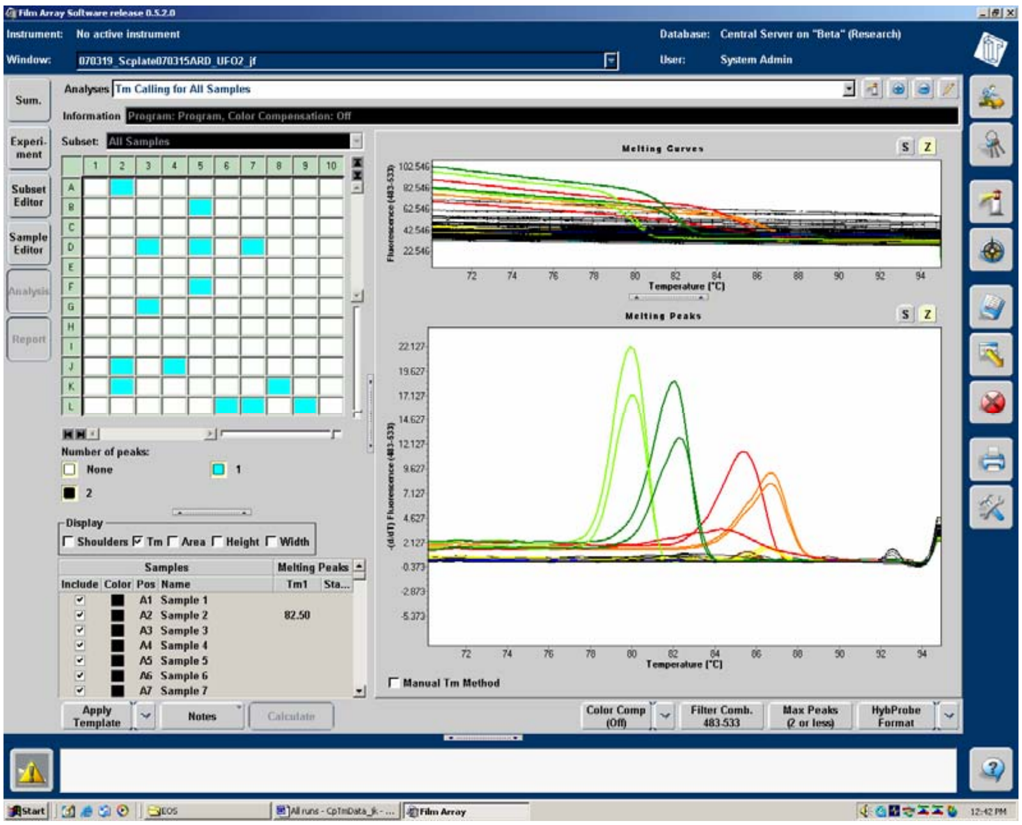
With the exception of the melt for ScD02 on the 05/03 run, all of the organism and control amplicon specific melts are very close in temperature between runs

The table below shows the color-coding of the amplicons used in these runs. The Cp and Tm data refer to the data from the run at the EOS demonstration by Idaho Technology on May 7 (data shown on page 8).

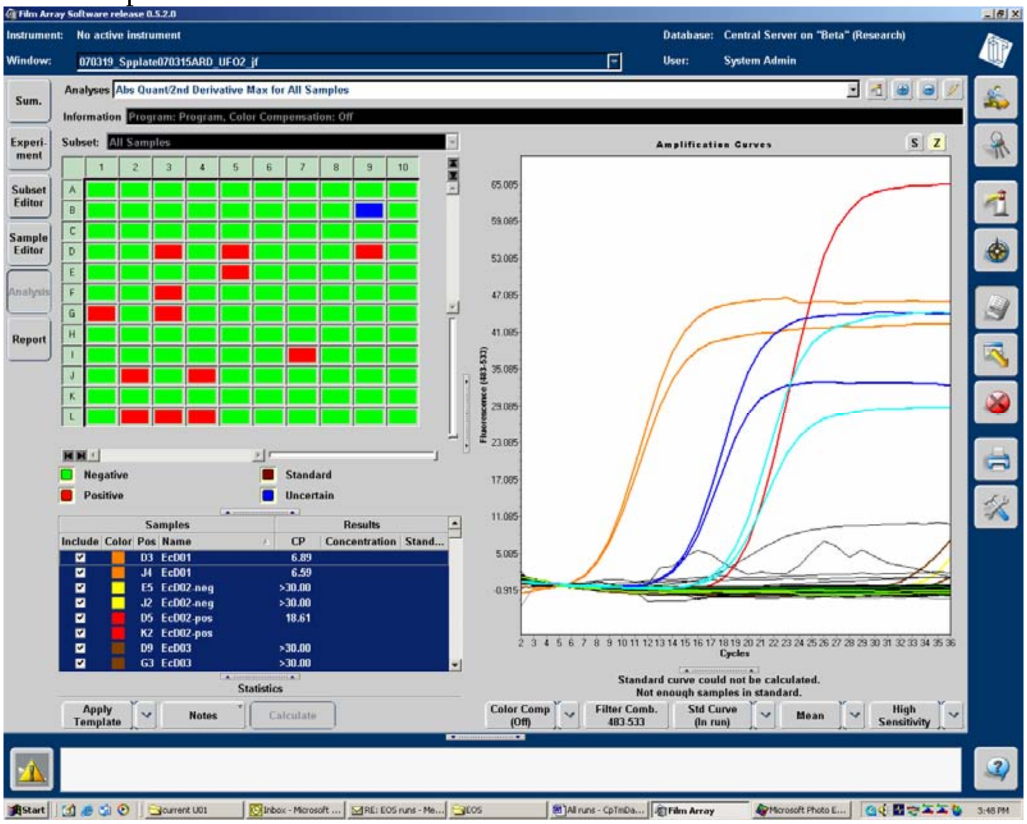
The EcD03 assay was not used in every pouch.

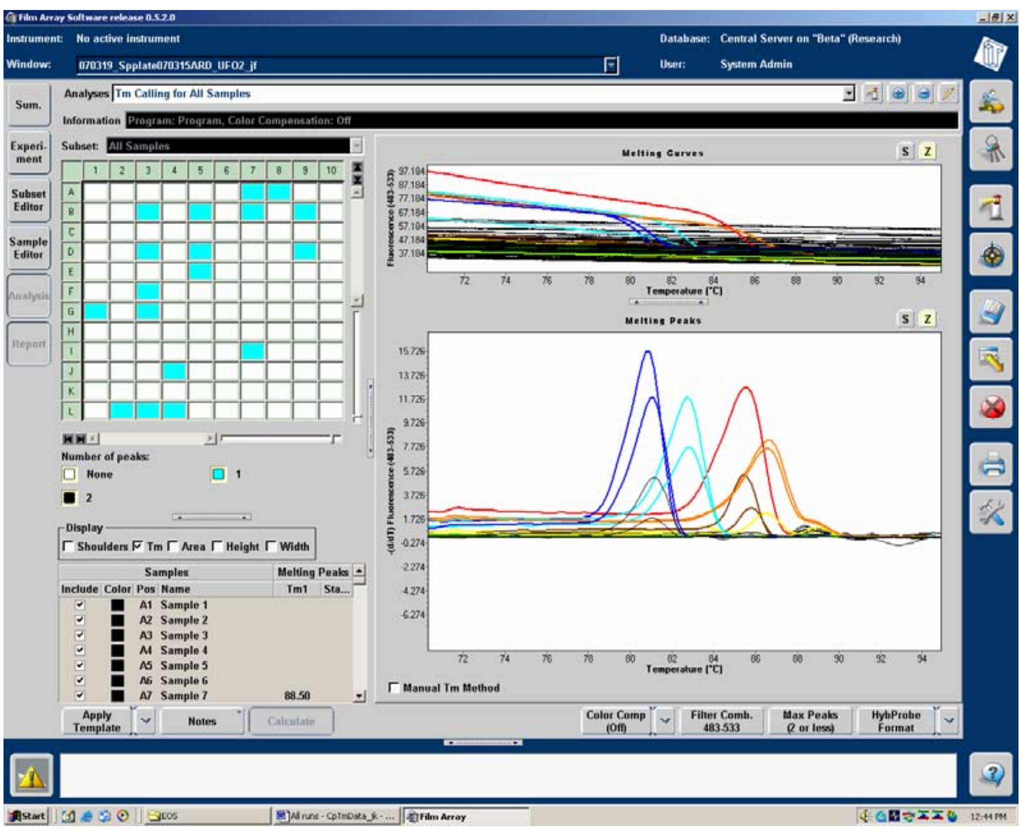
	Name	Graph Color	CP	Tm1
PCR1 control	EcD01	Orange	>25.0	85.8
	EcD01	Orange	>25.0	85.6
Cross talk control	EcD02-neg	Yellow		
	EcD02-neg	Yellow		
PCR2 control	EcD02-pos	Red	18.6	84.5
	EcD02-pos	Red	18.5	84.5
Dilution control	EcD03	Brown		80.2
	EcD03	Brown		80.4
S. cerevisiae specific amplicons	ScD05	Dark Green	15.8	81.0
	ScD05	Dark Green		81.1
	ScR03	Light Green	17.3	79.0
	ScR03	Light Green	17.7	79.0
S. pombe specific amplicons	SpD02	Dark Blue		
	SpD02	Dark Blue		
	SpR05	Light Blue		
	SpR05	Light Blue		

070319 Sc PCR and Melt curves

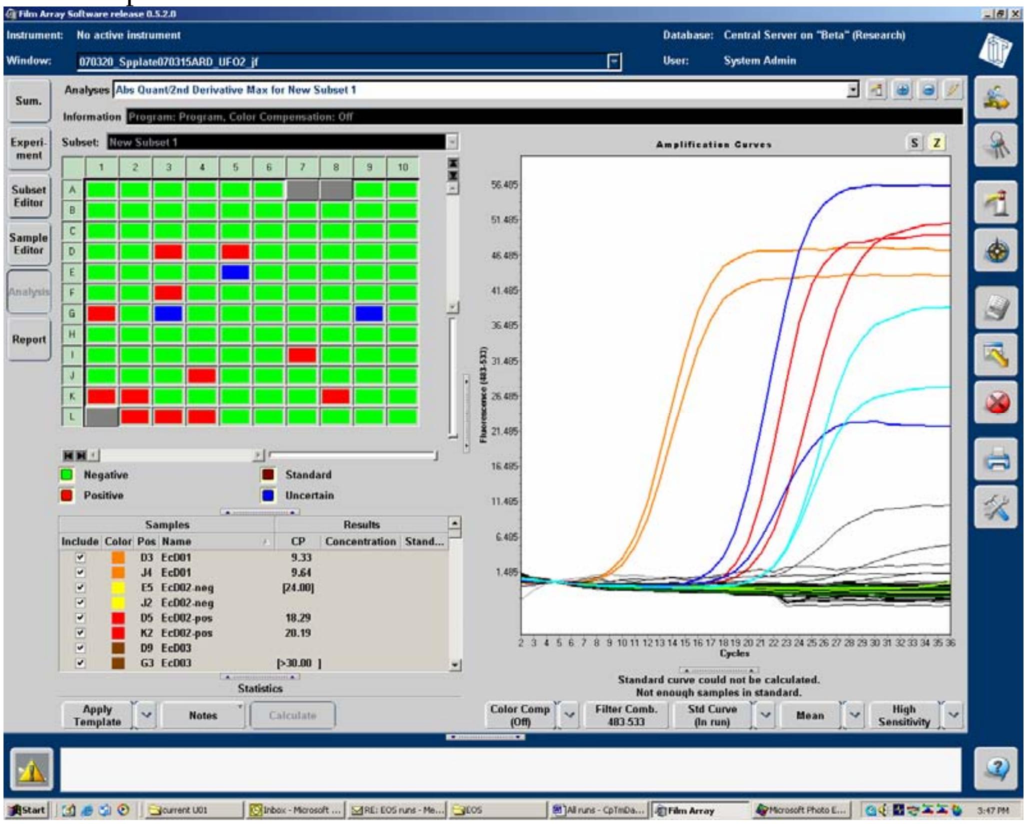


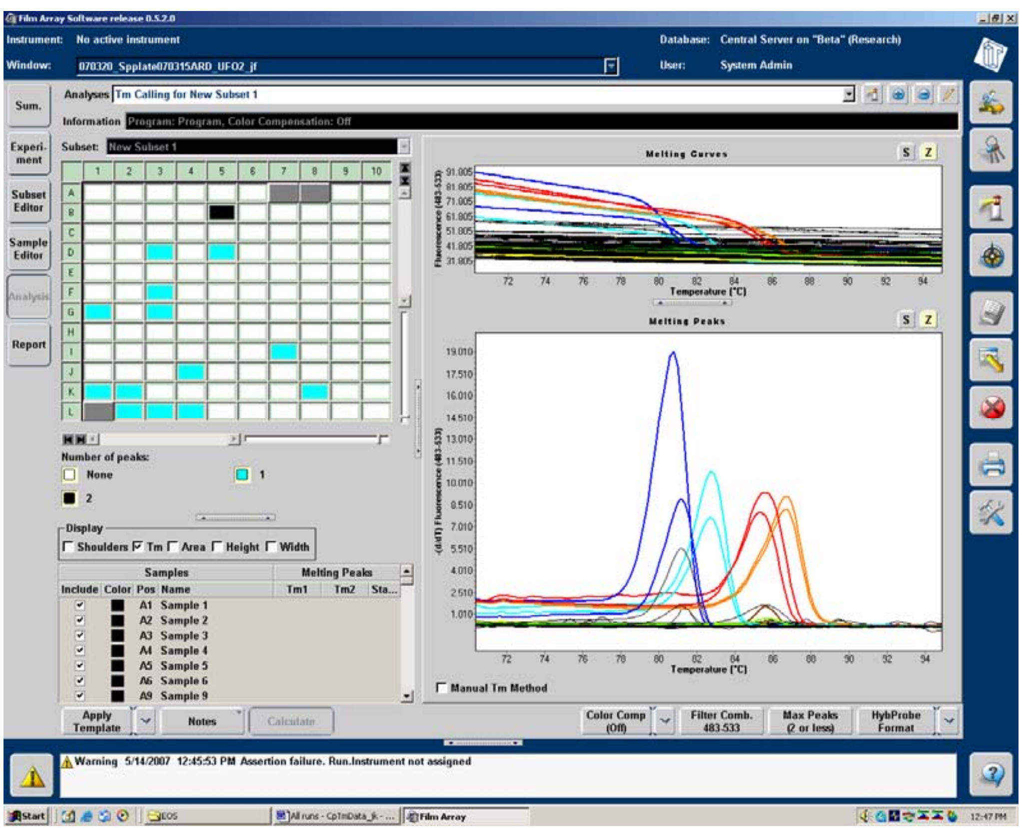
070319 Sp PCR and Melt curves





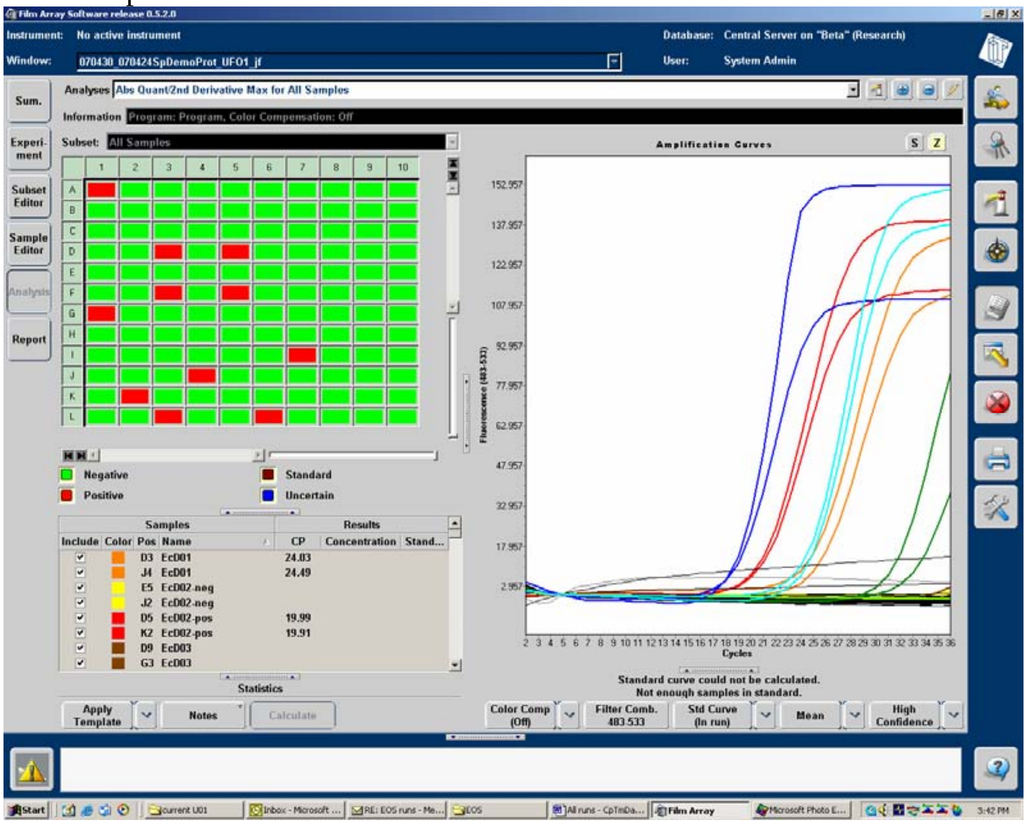
070320 Sp PCR and Melt curves



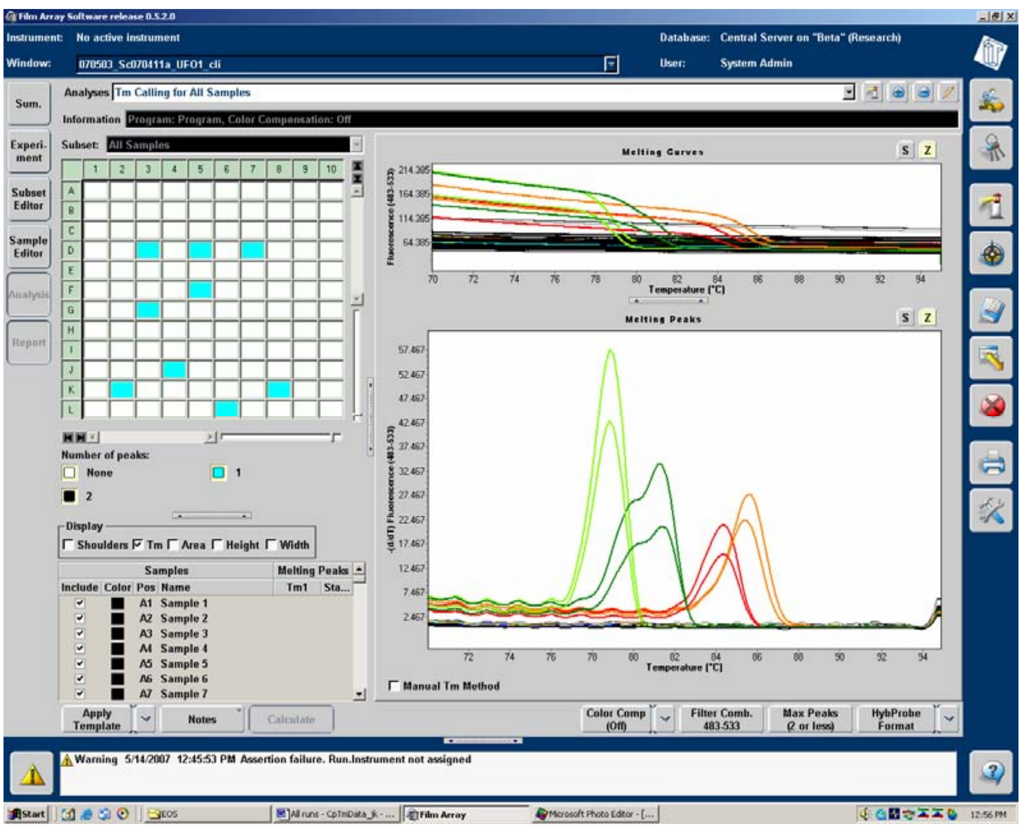


070321 Sc PCR and Melt curves

070430 Sp PCR and Melt curves



070503 Sc PCR and Melt curves



070507 Sc PCR and Melt curves

AKONNI BIOSYSTEMS
STATEMENT OF WORK
BAA 06-1 A7KM Log #19
26 September 2006

BASE PROGRAM:

There are 3 major steps which describe the protocol and operational capability of the diagnostic test:

#1) sample preparation;
#2) PCR amplification;
#3) microarray detection.

Operational Capability & Deliverables in 12 Months:

For the prevalidation study, the contractor will deliver a microfluidic device which incorporates operational capabilities #2 and #3 described above. Contractor will deliver a sample preparation kit with each microfluidic card test. Contractor will deliver a prototype instrument capable of running the microfluidic card (fluidic control, PCR thermal cycling, microarray incubation, microarray scanning). Contractor will deliver a microarray capable of identifying 10 pathogens and as appropriate the microarray will include multiple signatures per pathogen and multiple positive and negative controls; i.e. up to 100 features per array. Targets are specified by the customer in the document: “EOS ACTD Advanced Diagnostic Pre-Validation Test Plan”; targets are listed below in Table 1. The test will be designed to meet the following user requirements (as described in the document: “EOS ACTD Advanced Diagnostic Pre-Validation Test Plan”:

- Capability of identifying 10 upper respiratory pathogens (shown below in Table 1)
- 10^3-10^7 CFU or PFU/mL detection range
- Less than a 4 hour detection protocol (time to detect including sample prep)
- CV% < 10% (n=8)
- Operated by less than 2 technologists
- Footprint <150 ft^2

Table 1. Target positive and negative panels for pre-validation study

Positive Panel	Negative Panel
Adenovirus	Mycoplasma orale
Bordetella pertusis	Staphylococcus aureus
Chlamydia pneumoniae	HSV-1
Human Parainfluenza	Neisseria species
Influenza A	Enterobacteriacea
Influenza B	Proteus species
Mycoplasma pneumoniae	Coxsackievirus or Echovirus
Repiratory Syncitial Virus	Hemophilus influenzae
Coronavirus	Cells from human bronchial epithelium
Streptococcus species	Chlamydia trachomatis

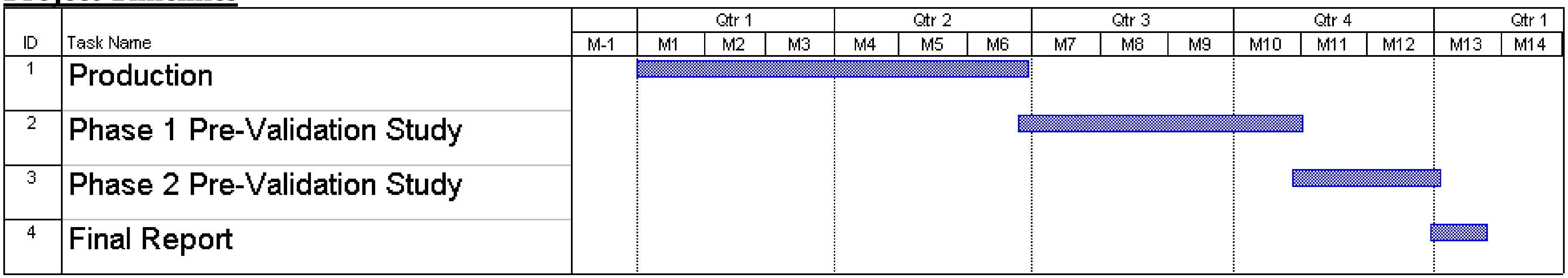
The major deliverable for this phase is a data package supporting successful completion of pre-validation under the boundaries described above. In summary, Akonni will deliver the following for this project: A) 1 controller instrument, B) 1320 microfluidic tests, C) standard operating procedure; including sample prep, D) operation manuals, E) spec sheets, and F) pre-validation data analysis, monthly/ final reports. A detailed deliverable table is shown below in Table 2.

Table 2: Table of Tasks and Deliverables

Task #	Task Description	POP	Deliverable # and Description	Due Date	Notes
1	Produce Tests & Auxiliary Material	M1-M10	1. Produce and deliver 1320 prototype microfluidic controlled microarray devices for use in the pre-validation study. 2. Supply instrumentation, hardware, software, and protocols for the performance of the pre-validation study.	Continuous	A total of 1320 devices (tests) will be produced for the pre-validation study (including deliverable 7)
2	Phase 1 Pre-validation Study	M6-M10	3. Deliver data (150 data points) demonstrating detection at 10^3, 10^4, 10^5, 10^6, 10^7 CFU or PFU/mL for each target pathogen from the positive panel (spiked into a swab sample)	End of M10	Positive panel from Table 1. Data delivered in the month 10 technical report. A total of 3 replicates per pathogen per concentration will be delivered (total of 3 X 10 X 5 = 150 data points)
2	Phase 1 Pre-validation Study	M6-M10	4. Deliver data (240 data points) demonstrating detection at 10^3, 10^5, 10^7 CFU or PFU/mL for each target pathogen from the positive panel (spike into a swab sample) with a CV% < 10% (n=8). Calculated results will include mean S.D. and CV%	End of M10	Positive panel from Table 1. Data delivered in the month 10 technical report. A total of 8 replicates per pathogen per concentration will be delivered (total of 8 X 10 X 3 = 240 data points)
2	Phase 1 Pre-validation Study	M6-M10	5. Deliver data (150 data points) demonstrating zero or minimal cross reactivity (no false positives) of the microarray when tested against the negative panel pathogens at 10^3, 10^4, 10^5, 10^6, 10^7 CFU or PFU/mL (spiked into a swab sample)	End of M10	Negative panel from Table 1. Data delivered in the month 10 technical report. A total of 3 replicates per pathogen per concentration will be delivered (total of 3 X 10 X 5 = 150 data points)
2	Phase 1 Pre-validation Study	M6-M10	6. Deliver a standard operating protocol used for the pre-validation testing	End of M10	Delivered in the month 10 technical report.
3	Phase 2 Pre-validation Study	M11-M12	7. Deliver 780 tests, instrumentation, hardware, software, and protocols for a repeat performance of the pre-validation study at AFIOH/ADL	M11	Delivered in month 11 to AFIOH/ADL
3	Phase 2 Pre-validation Study	M11-M12	8. Provide on-site training a technical support during the repeat performance of the pre-validation study at AFIOH/ADL	M11-M12	Delivered in months 11-12 to AFIOH/ADL
4	Final Report	M13	9. Demonstrate a less than 4 hour detection protocol including sample processing	End of M13	A protocol detailing time constraints will be delivered in the final report
4	Final Report	M13	10. Deliver an instrument specification sheet demonstrating a footprint <150 ft^2.	End of M13	A spec sheet will be delivered in the final report. The spec sheet will describe the instrument used to perform the pre-validation study (as opposed to some future instrument form factor).
4	Final Report	M13	11. Demonstrate that the test is operable by less than or equal	End of M13	The protocol detailing operator constraints will be delivered

			to 2 technologists		in the final report
4	Final Report	M13	12. Deliver a list of pathogens from the EOS ACTD supplied menu that were used in the pre-validation study	End of M13	Delivered in the final report.
4	Final Report	M13	13. Deliver raw data files for each pre-validation test run	End of M13	Delivered in the final report.
	Monthly Reports		Monthly Reports	Monthly	
	Financial Reports		Financial Reports	Monthly	

Project Timelines



Task 1: Production (6 Months)

• Design and produce 1320 microfluidic devices for use in the validation study. The test will have biomarkers for the pathogens described in column one of Table 1. The test will have built in positive and negative controls.
• Contractor will deliver a microarray capable of identifying the positive pathogen panel described in Table 1. Targets and biomarkers will be species-specific for the organisms listed on Table 1 with the approval of the EOS molecular biologist.

Table 1. Target positive and negative panels for pre-validation study

Positive Panel	Negative Panel
Adenovirus	Mycoplasma orale
Bordetella pertusis	Staphylococcus aureus
Chlamydia pneumoniae	HSV-1
Human Parainfluenza	Neisseria species
Influenza A	Enterobacteriacea
Influenza B	Proteus species
Mycoplasma pneumoniae	Coxsackievirus or Echovirus
Repiratory Syncitial Virus	Hemophilus influenzae
Coronavirus	Cells from human bronchial epithelium
Streptococcus species	Chlamydia trachomatis

• **Genetic-array Production**: Design, fabricate and perform pre-validation testing of a microfluidic microarray test capable of controlling an Akonni designed nucleic acid based array. Develop target sequences, primer and probe design, assay protocol. Fabricate microfluidic microarray tests. Akonni will design and produce the on-chip PCR gel-drop microarray and work with the USAF to identify and select pathogen specific genes and primers and probes sequences to detect the high-priority pathogens. We will prioritize this list in consultation with the Program Manager when the project begins. For each of the selected pathogens up to five specific gene targets will be selected for assay development. Each on-chip PCR gel-drop microarray will have each gene assay for the selected pathogens in triplicates. The RNA-virus assays may require a separate incubation step to perform the reverse transcription step (this will be accounted for and built into the fluidic card design). The total number of gel-drop elements on a microarray card will be sufficient to identify the positive panel pathogens listed in Table 1 and detailed in the document: “EOS ACTD Advanced Diagnostic Pre-Validation Test Plan” (we estimate this number to be up to 100 elements). The microarray can be expanded for more pathogens in the future.

• **Produce 1 controller units** for use in the Base Program pre-validation study. **Goals**: Engineer current controller instrument to meet user requirements. This will include the integration and component selection of fluidic pumps, optical source/detector, heater and controller software/GUI. The unit has been developed and is available as a commercial instrument; this task will focus on configuration and engineering to meet user requirements for the pre-validation study (i.e. < 150 ft^2). Produce instruments with the current manufacturing/sourcing capabilities.

Task 2: Phase 1 Pre-Validation Study (4 Months)

The contractor will deliver data (540 data points) for describing the performance of the tests. The pre-validation study will occur at Akonni labs under BSL2 conditions. All organisms will be acquired from the ATCC. The test matrix will include the testing of specified targets and near neighbors as described in Table 1. Details of the deliverable data are provided in Table 2. Specifically, the contractor will test microarrays with the positive and negative panels to statistically demonstrate the following requirements:

- 10^3-10^7 CFU or PFU/mL detection range
- Less than a 4 hour detection protocol (time to detect including sample prep)
- CV% < 10% (n=8)
- Operated by less than 2 technologists

Task 3: Phase 2 Pre-Validation Study (2 Months)

The contractor will deliver the system to the customer-specified location (i.e. AFIOH ADL). The contractor will provide an onsite field rep for training and the pre-validation study. The training program is a 2 week program in which the contractor personnel will:

- Train the customer to run the diagnostic system.
- Assist the customer in optimizing microfluidic and assay conditions.
- Assist in generating initial data with genomic material (for training purposes).
- Finalize the SOP which will include a full protocol including material handling and sample preparation steps.
- Finalize decision logic for array readout and communicating results to the customer.

The contractor will deliver 780 tests for this study which represents a replicate study of deliverables 4 and 5 shown in Table 2 (and as described in the document: “EOS ACTD Advanced Diagnostic Pre-Validation Test Plan”. In addition to the instrument supplied to the ADL (deliverable 7, Table 2), the contractor will supply an additional loaner unit for comparative testing during the validation phase. One of the diagnostic units (System 1) will be run by the contractor field rep and the other unit (System 2) will be run by the customer lab technician. Each sample will be run on both systems as a comparative measure.

Task 4: Final Report (30 days)

• Data analysis and final report

SOLICITATION, OFFER AND AWARD

1. THIS CONTRACT IS A RATED ORDER UNDER DPAS (15 CFR 700) | RATING | PAGE 1 OF PAGES 31

2. CONTRACT NO: FA7014-06-C-0030
3. SOLICITATION NO.
4. TYPE OF SOLICITATION [ ] SEALED BID (IFB) [ ] NEGOTIATED (RFP)
5. DATE ISSUED
6. REQUISITION/PURCHASE NO.: F1ATD46172A400

7. ISSUED BY — CODE FA7014
FA7014- AIR FORCE DISTRICT OF WASHINGTON
110 LUKE AVE SUITE 200 BLDG 5681
BOLLING DC 20032-0305
TEL:
FAX:

8. ADDRESS OFFER TO (If other than Item 7) — CODE
See Item 7
TEL:
FAX:

NOTE: In sealed bid solicitations "offer" and "offeror" mean "bid" and "bidder".

SOLICITATION

9. Sealed offers in original and ______ copies for furnishing the supplies or services in the Schedule will be received at the place specified in Item 8, or if handcarried, in the depository located in ______ until ______ (Hour) local time ______ (Date)

CAUTION - LATE Submissions, Modifications, and Withdrawals: See Section L, Provision No. 52.214-7 or 52.215-1. All offers are subject to all terms and conditions contained in this solicitation.

10. FOR INFORMATION CALL: A. NAME | B. TELEPHONE (Include area code) (NO COLLECT CALLS) | C. E-MAIL ADDRESS

11. TABLE OF CONTENTS

OFFER (Must be fully completed by offeror)

NOTE: Item 12 does not apply if the solicitation includes the provisions at 52.214-16, Minimum Bid Acceptance Period.

12. In compliance with the above, the undersigned agrees, if this offer is accepted within ______ calendar days (60 calendar days unless a different period is inserted by the offeror) from the date for receipt of offers specified above, to furnish any or all items upon which prices are offered at the price set opposite each item, delivered at the designated point(s), within the time specified in the schedule.

13. DISCOUNT FOR PROMPT PAYMENT (See Section I, Clause No. 52.232-8): Net 30 Days

14. ACKNOWLEDGMENT OF AMENDMENTS (The offeror acknowledges receipt of amendments to the SOLICITATION for offerors and related documents numbered and dated):

AMENDMENT NO.	DATE	AMENDMENT NO.	DATE

15A. NAME AND ADDRESS OF OFFEROR — CODE 3YNV1 — FACILITY
AKONNI BIOSYSTEMS INC
CHARLES DAITCH
9702 WOODFIELD COURT
NEW MARKET MD 21774-2912

16. NAME AND TITLE OF PERSON AUTHORIZED TO SIGN OFFER (Type or print): CHARLES DAITCH / PRESIDENT

15B. TELEPHONE NO (Include area code): 3015247867

15C. CHECK IF REMITTANCE ADDRESS IS DIFFERENT FROM ABOVE - ENTER SUCH ADDRESS IN SCHEDULE. [ ]

17. SIGNATURE: CDaitch

18. OFFER DATE: 9/28/06

AWARD (To be completed by Government)

19. ACCEPTED AS TO ITEMS NUMBERED
20. AMOUNT: $876,060.00
21. ACCOUNTING AND APPROPRIATION: See Schedule

22. AUTHORITY FOR USING OTHER THAN FULL AND OPEN COMPETITION: [ ] 10 U.S.C. 2304(c)( ) [ ] 41 U.S.C. 253(c)( )

23. SUBMIT INVOICES TO ADDRESS SHOWN IN (4 copies unless otherwise specified) — ITEM: Section G

24. ADMINISTERED BY (If other than Item 7) — CODE
See Item 7

25. PAYMENT WILL BE MADE BY — CODE F67100
DEFENSE FINANCE AND ACCOUNTING SERVICE
DFAS-LIMESTONE
ATTN: DFAS-BAASD/CC
P.O. BOX 369020
COLUMBUS OH 43236-9020

26. NAME OF CONTRACTING OFFICER (Type or print): Gretchen Clohessy
TEL: 703·681·7034 EMAIL: gretchen.clohessy@bolling.af.mil

27. UNITED STATES OF AMERICA: Gretchen B Clohessy (Signature of Contracting Officer)

28. AWARD DATE: 28 Sep 06

IMPORTANT - Award will be made on this Form, or on Standard Form 26, or by other authorized official written notice.

Cepheid GeneXpert

Cepheid

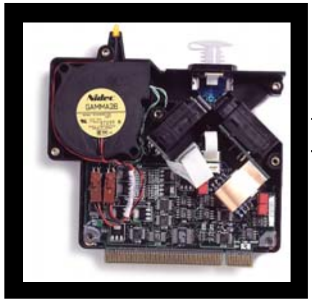
Sample in. Answer out.™

DNA test results –
“where and when they are needed”

Cepheid Technology

- Platforms
- Cartridges
- Reagents
- Protocols and Controls

Cepheid Platforms



I-CORE module

SmartCycler Instrument

GeneXpert Instrument

GX module

BDS

Platforms
GeneXpert Design Approach

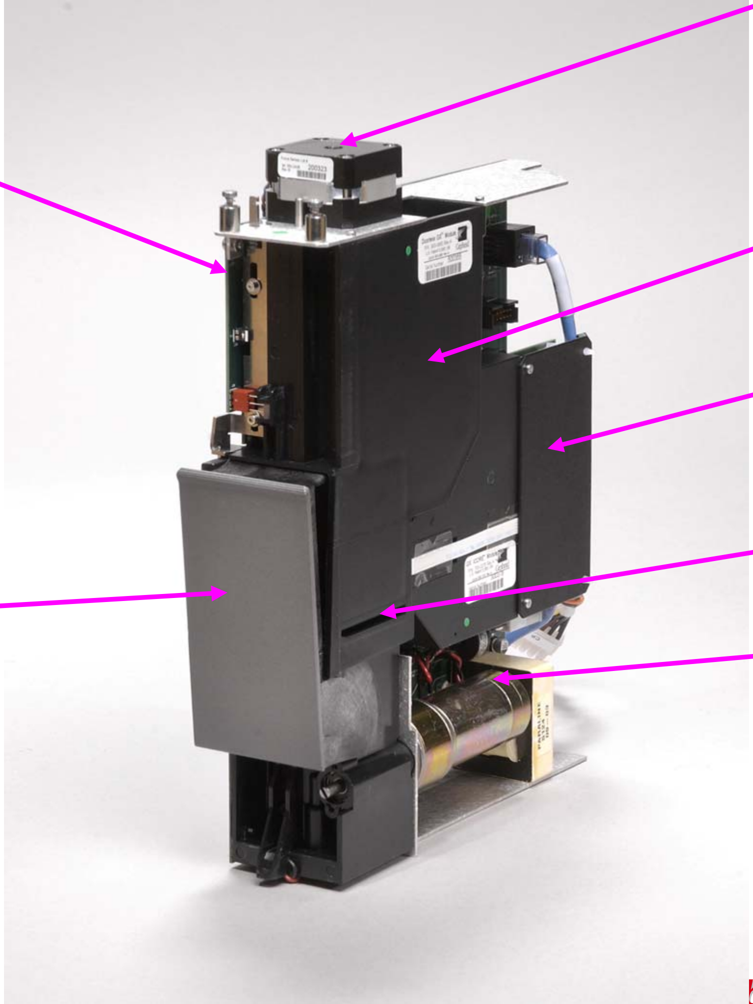
- Employ multiplex, rapid real-time PCR technique
  - Closed reaction tube, no opening required for reading
  - 4-colors for multiplexing current (6 in prototype)
- Utilize proprietary dried reagent technology for ambient temp stability and precision of microfluidics-based reconstitution
- Utilize families of cartridges to address wide range of applications (bacteria, viruses, fungi, cellular RNA, DNA, qual. & quant)
- No wet interface between instrument and cartridge to eliminate carry-over

CEPHEID CONFIDENTIAL – Not for distribution

Platforms
GeneXpert Design Approach

- Integrated ultrasonics for rapid lysis of bacteria and cells
  - Unique filter capture/concentration technique
- Total internal control of reagent system –
  - No separate external pos or neg controls required
  - Total process control
- Smart fluidics
  - Encoded sw-driven motors for random access valving and integral hydraulic drives
  - Advanced microfluidics technologies to enable complex sample prep processing protocols (many milliliters down to 10 microliters)
- Automated data reduction and results interpretation

Platforms
Assembled GeneXpert Module



GeneXpert® Platform

Benefits of a unique integrated closed system

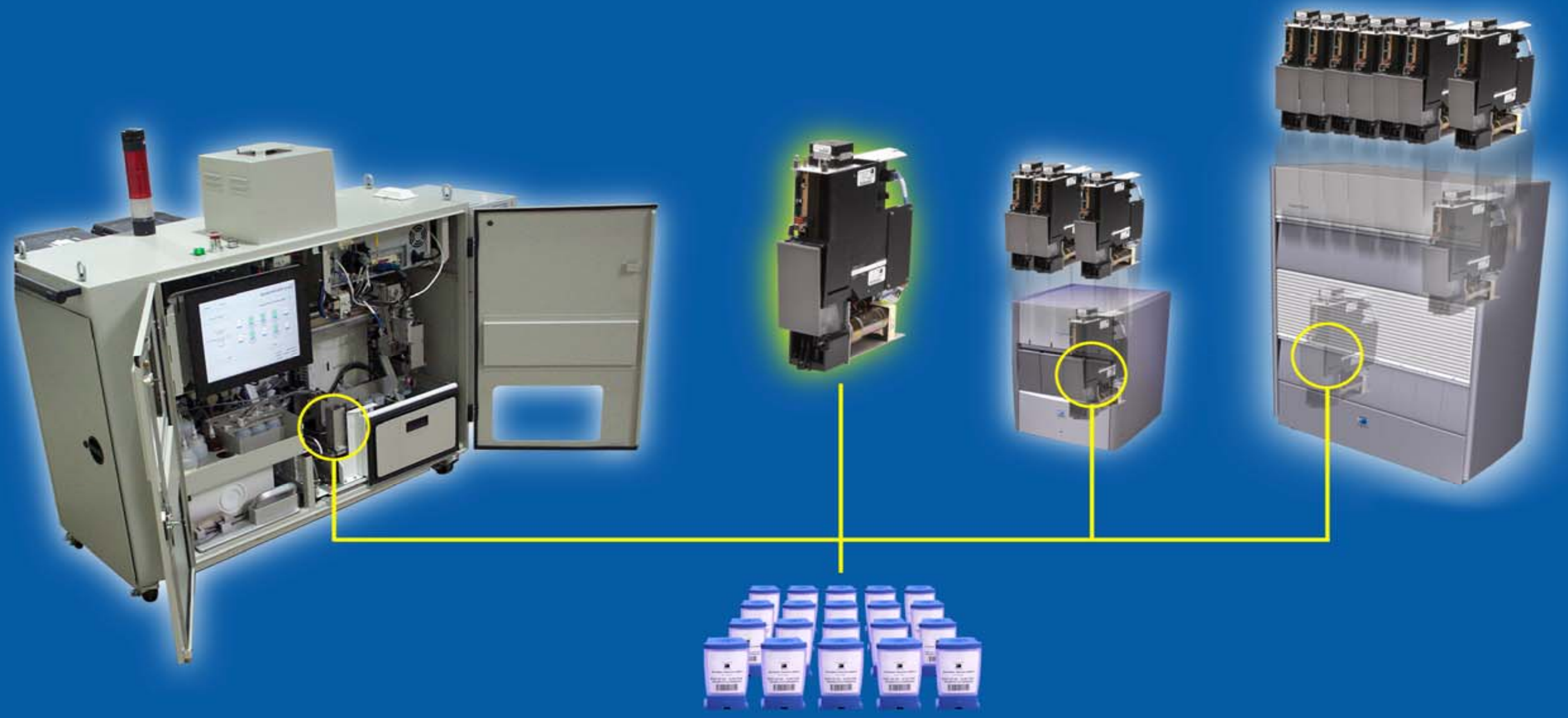
- Proven specificity
  - ➢ No false positives recorded in nearly 4.5 million anthrax tests to date
- Extraordinary sensitivity
- Built-in Reflex testing

GX Module GX I GX IV GX XVI

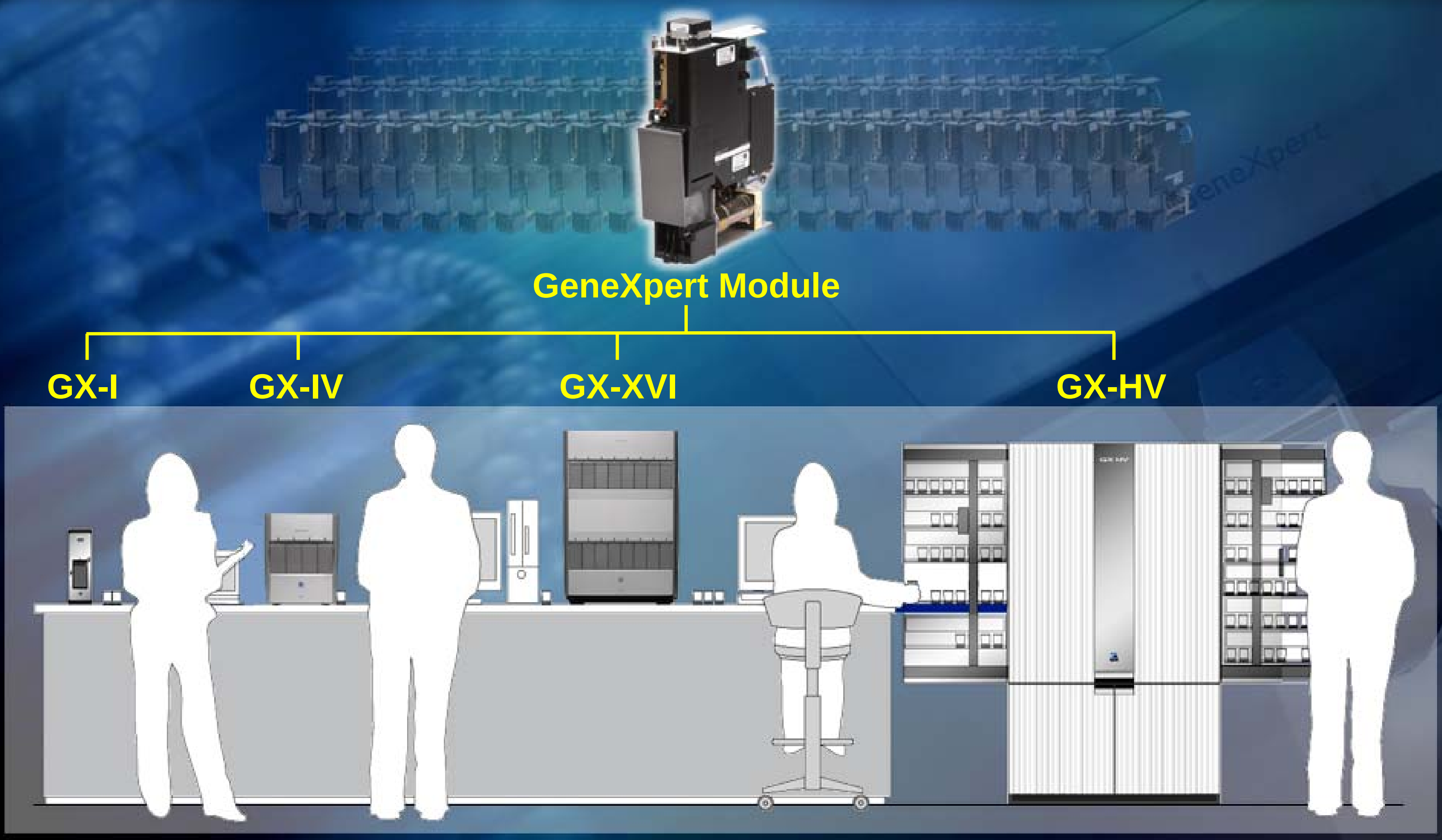
CEPHEID CON

Cepheid Sample in. Answer out.™

The Cepheid Solution



GeneXpert® Platform Strategic Reach



The Cepheid Solution

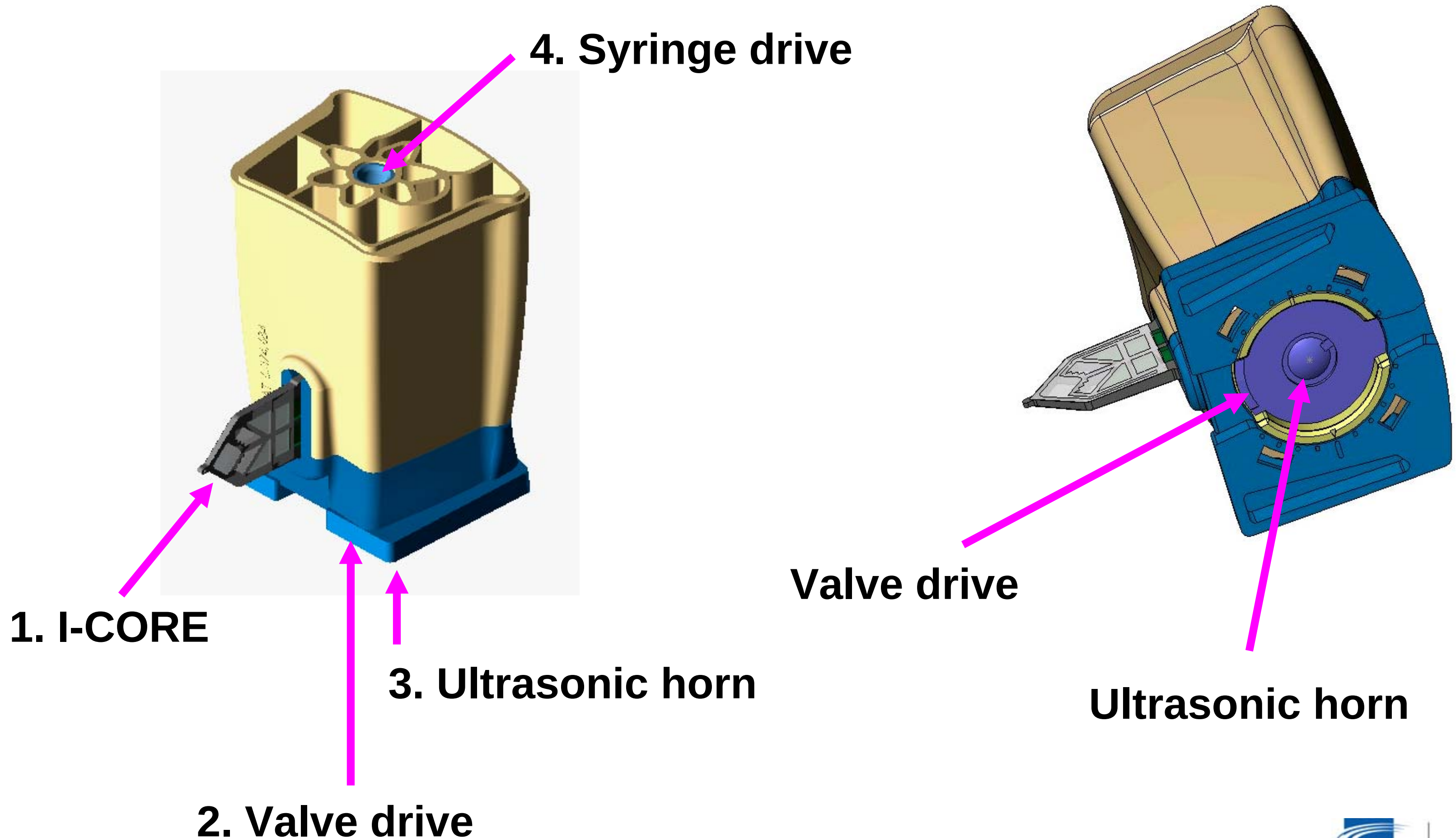
GeneXpert technology represents a paradigm shift in automation

- •Marketability
  - •Reference Labs
  - •Hospital Labs
  - •Doctor's Office
- •Ultimate specificity
  - •Anthrax test in use at the USPS has not had a single false positive from >4.5 million tests
- •Ultimate sensitivity
  - •Assay cartridge design allows use of nested PCR in a totally closed environment
- •Ultimate ease of use
  - •Sample preparation is integrated with amplification and detection

Platforms
Next Generation I-CORE module : 6 Colors

- Goals
  - Maintain rapid cycling capability
  - 6 discrete color channels with possibility of additional colors at off-axis
  - Improvements in optical performance
  - Same I-CORE footprint and electronic packaging

Platforms
Cartridge – Instrument Interfaces



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Platforms
GX Cartridge Operation

Fluid Aspirate

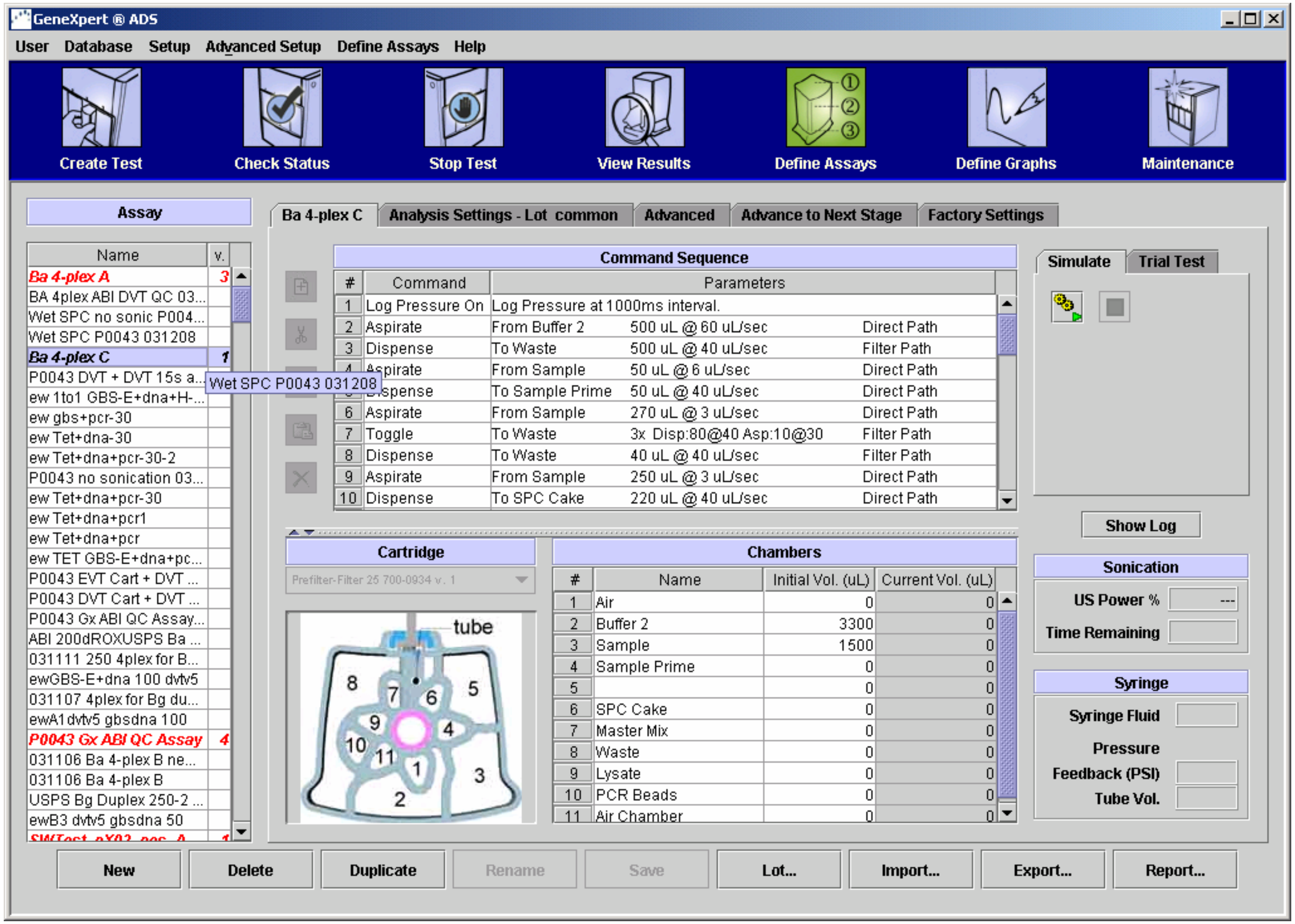
Reagent Mixing

Filtration

Ultrasonic Lysis

Tube Fill for PCR

Platforms
Developers Software



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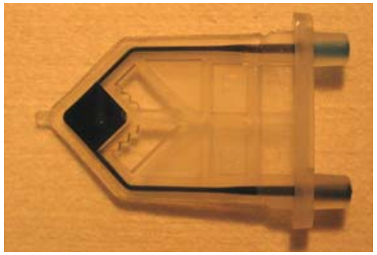
Cepheid

Sample in. Answer out.™

Cartridges
Exploded View

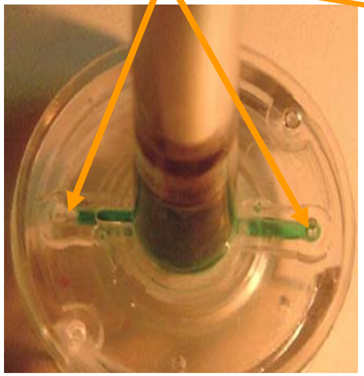
Molded Prefilter
bottom of chamber 3

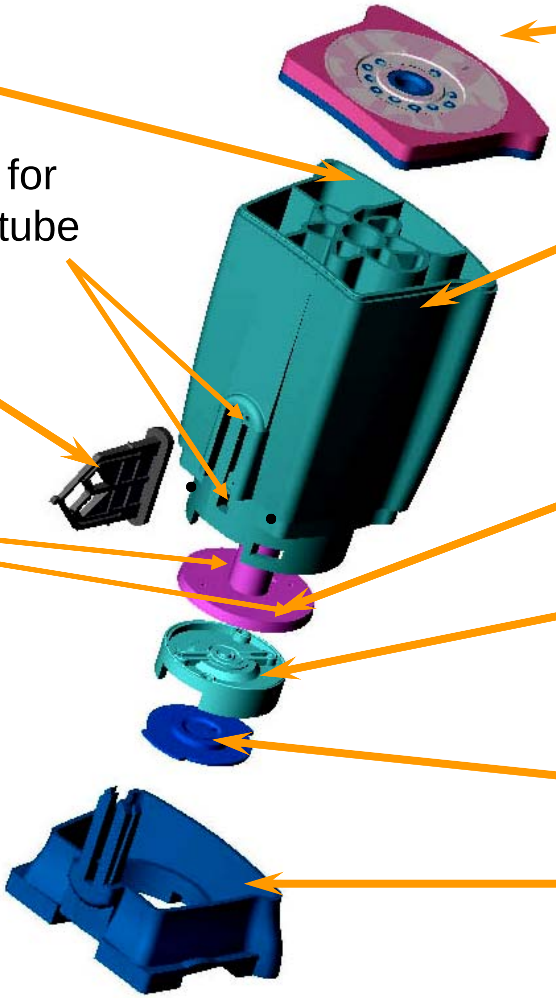


PCR
Tube

Ports for
PCR tube

Valve body Ports





Lid

Cartridge Body
(11 Fluid Chambers and
overmolded gasket)

Syringe Barrel

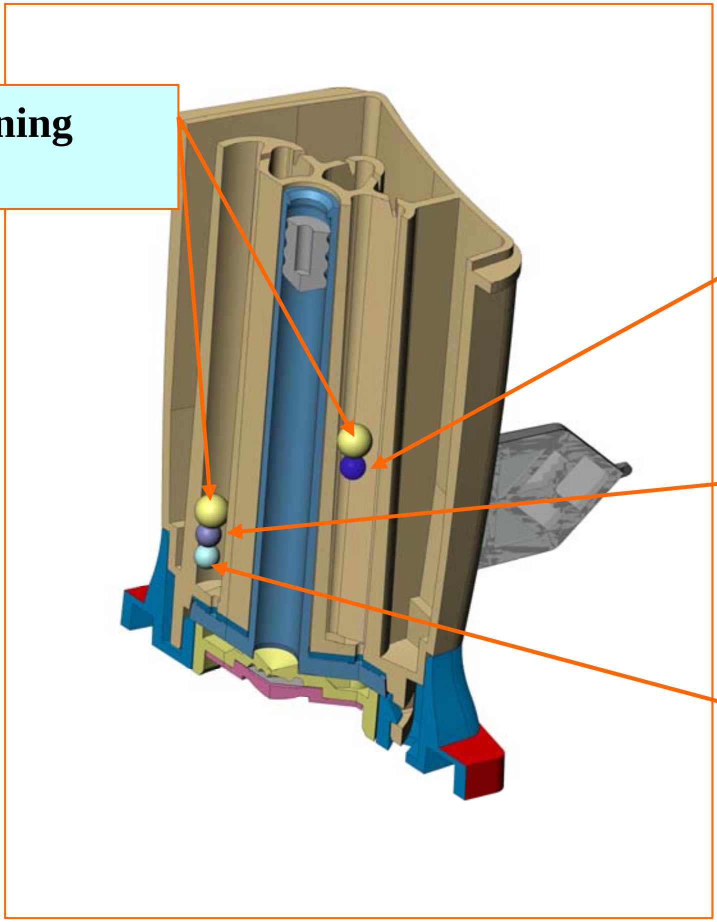
Rotary Valve/Filter and
Ultrasonic Lysis or
SPBRegion

Cap/Ultrasonic Interface

Cartridge Foot

Cartridges

Reagent Beads in Cartridge



Sample preparation bead:

Sample prep internal control; excipients

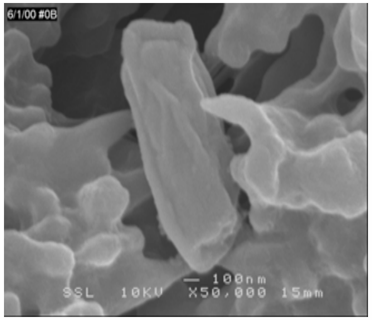
Enzyme reagent bead:

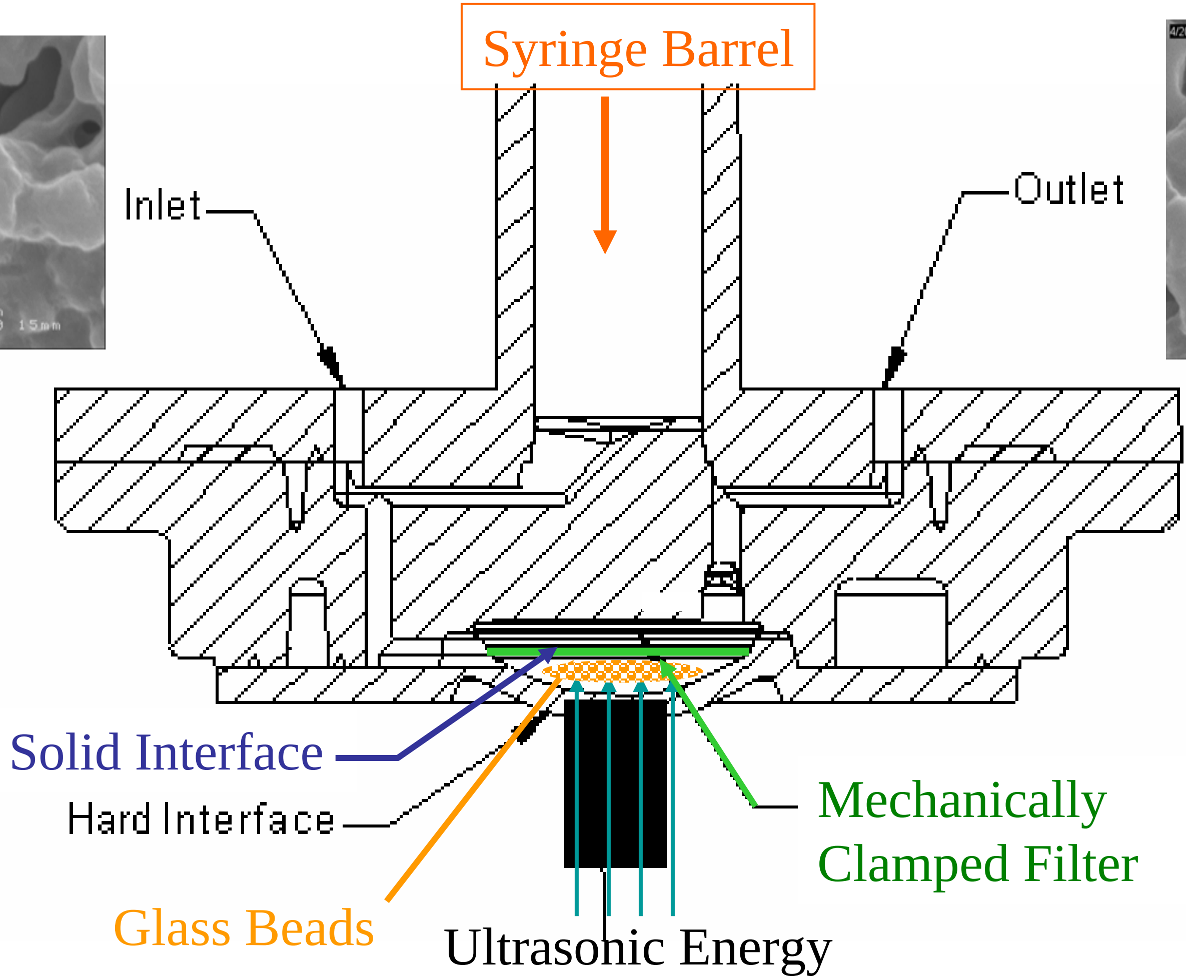
Hot start Taq polymerase; dNTPs; Hepes buffer; BSA; Mg 2+; salts; excipients

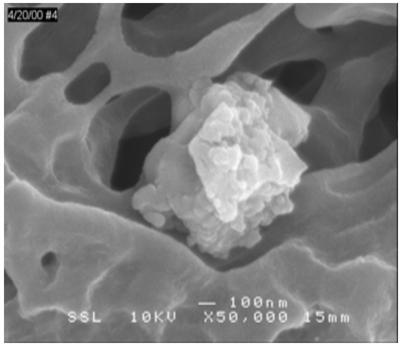
Target-specific reagent bead:

PCR primers; PCR probes; internal control template; Hepes buffer; Mg2+; excipients

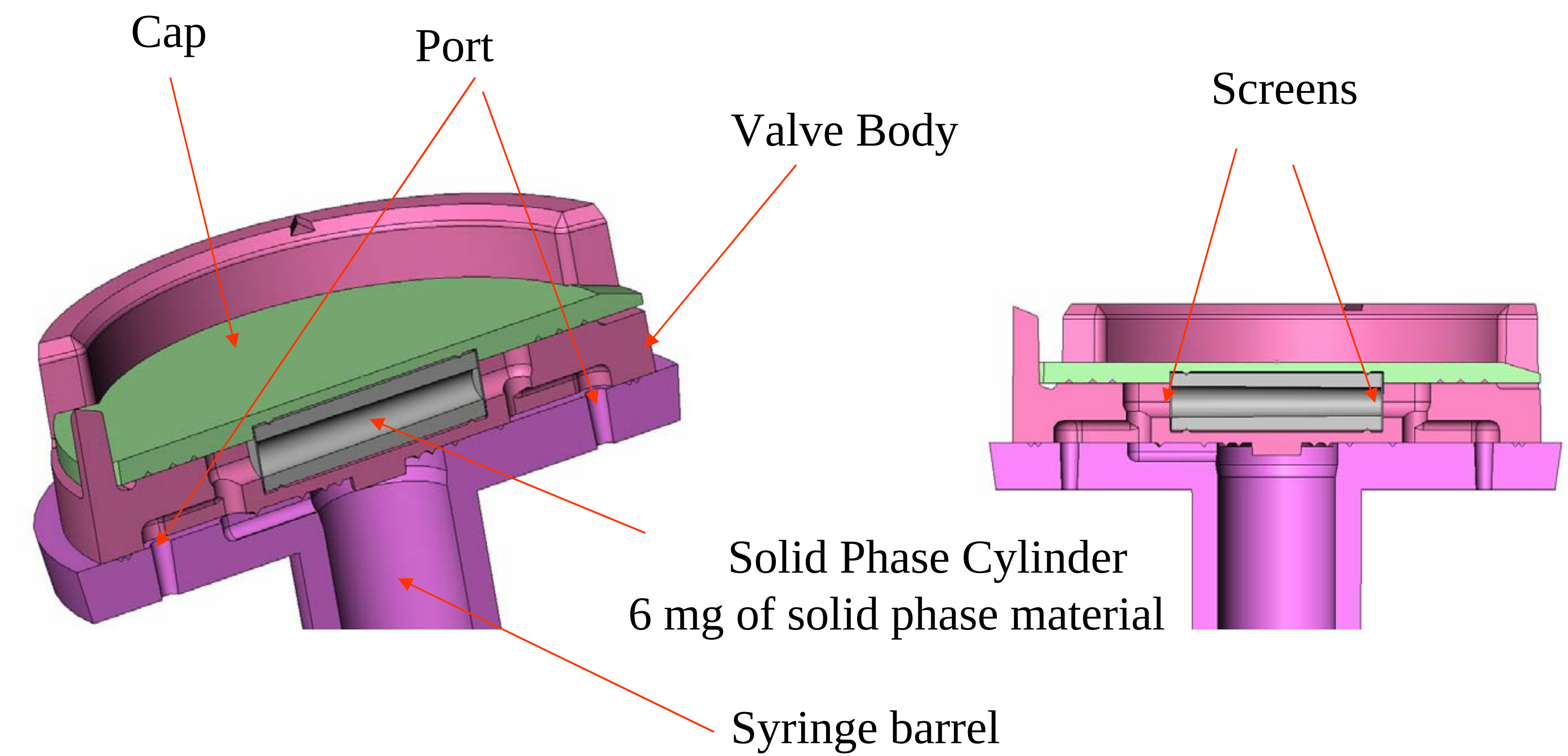
Cartridges
Filter Capture Valve Body Assembly







Cartridges
Valve Body: Solid Phase Bolus (SPB)

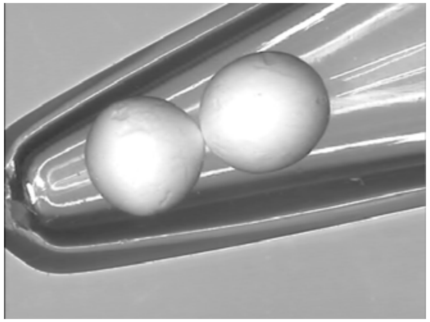


Reagents

- Sequences
  - Externally sourced from academic collaborators or industrial/biotech or government (USAMRIID) partners
  - Internally designed
  - FTO process (IC Bio search; in-house claims evaluation; licensing)
- Dyes and Quenchers
  - Cepheid specific dyes and quenchers
- Enzymes
  - Licensed vendors for a variety of enzymes (Taq; inhibitors; reverse transcriptase)
- Real-time PCR
  - TaqMan, Beacons, and Scorpions

Reagents

- Dry Beads
  - Proprietary bead formulation for leading edge performance, stability, dissolution rates
  - Separate critical components into formulations based on stability requirements
- Modified Bases and additives
  - Cepheid modified bases
  - AB modified bases
  - PA
- Primer and Probe design tools
  - GC clamps
  - Visual OMP; AB-proprietary programs
  - MGB's (ABI)



Reagents

- Internal control system assures validity and accuracy of each test
  - Probe check
    - Cartridge and reagent functionality/integrity
  - Internal control
    - Inhibition and reagent functionality
  - Sample prep control
    - Sample prep processing effectiveness
  - Combined SPC/IC

Cepheid Developed SmartCycler Assays

Product	Regulatory Classification	Status
B. pertussis	ASR*	Market
B. pertussis/parapertussis	ASR*	Market
HSV Non-typing	ASR*	Market
HSV Typing	ASR*	Market
Enterovirus	ASR*	Market
Parvovirus B19	ASR*	Market
TACSTD1	ASR*	Market
GUS	ASR*	Market
Spa (*Staphylococcus aureus*)	ASR*	Market
mecA	ASR*	Market
Influenza (A/B)	ASR*	Market
Respiratory syncytial virus (RSV)	ASR*	Market
Mycoplasma pneumoniae	ASR*	Market
Norovirus	ASR*	Market

***Analyte Specific Reagents (ASR).** Analytical and performance characteristics are not established. ASRs can only be sold to clinical laboratories regulated under the CLIA of 1988 as qualified to perform high complexity testing under 42 CFR part 493 or under VHA directive 1106.

Cepheid IVD SmartCycler Assays

Product	Regulatory Classification	Status
Group B strep	510(k)/CE-IVD	Market
CMV	CE-IVD	Market
VZV	CE-IVD	Market
EBV	CE-IVD	Market
HBV	CE-IVD	Market

Cepheid GeneXpert Assays

Product	FDA Classification	Status
Anthrax	N/A	Market
Anthrax/Pestis/Tularensis	N/A	Market
BCR/ABL	RUO*/CE-IVD	Market
GBS	510(k)/CE-IVD	Market
Enterovirus	510(k)/CE-IVD	Market
MRSA	510(k)/CE-IVD	Submitted to FDA
MRSA/MSSA	510(k)/CE-IVD	Development
Factor 2/5	510(k)/CE-IVD	Development
TB/rif resistance	CE-IVD	Development
Flu (AI)	510(k)/CE-IVD	Development
Sepsis Panels		
Gram Positive	PMA/CE-IVD	Development
Gram Negative	PMA/CE-IVD	Development
Fungal	PMA/CE-IVD	Development

*RUO. For Research Use Only. Not for use in diagnostic procedures.

Sample in. Answer out.™

Cepheid GeneXpert Assays

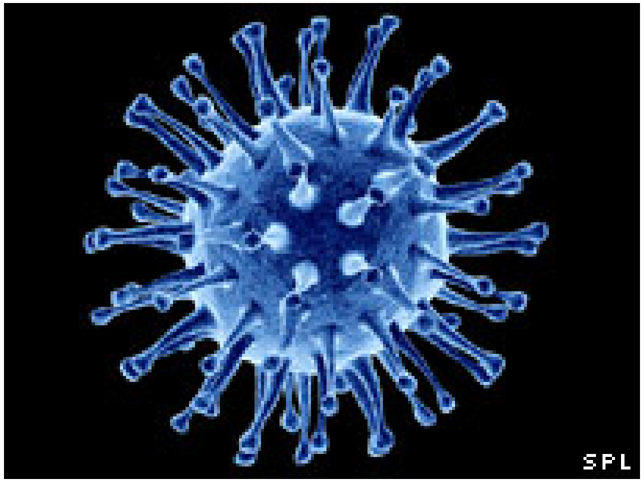
Product	Sample Type	Time to Result
Anthrax	Environmental Spores	37 minutes
Anthrax/Pestis/Tularensis	Environmental Spores/Bacteria	37 minutes
BCR/ABL	RNA from Whole Blood	140 minutes
GBS	Bacteria from Swabs	75 minutes
Enterovirus	RNA Virus from CSF	120 minutes
MRSA	Bacterial DNA from Nasal Swabs	73 minutes
MRSA/MSSA	Bacterial DNA from “wound” Swab	<60 minutes
Factor 2/5	DNA from Whole Blood	37 minutes
TB/rif resistance	DNA from Sputum	~60 minutes
Flu (AI)	RNA Virus from Throat/NP Swabs	~35 minutes
Sepsis Panels		
Gram Positive	Bacteria from Whole Blood	~60 minutes
Gram Negative	Bacteria from Whole Blood	~60minutes
Fungal	Bacteria from Whole Blood	~60 minutes

Cepheid GeneXpert
Infectious Disease Partnerships

- Flu A/B including H1, H3, and H5
  - CDC grant, December 2006 – May 2009
- M. tuberculosis and rifampin resistance
  - FIND grant, May 2006 – April 2009
  - NIAID SBIR Phase 2 grant, August 2006 – July 2008
- Sepsis Pathogen and Antibiotic Resistance Panels
  - bioMerieux, February 2007-completion
- BIAD-2--DHS

Xpert Flu

Sample in. Answer out.™



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Xpert Flu Product Design Goals

- Use PCR to detect seasonal human influenza viruses
  - Differentiate influenza A H5N1 from seasonal human influenza viruses
- Time to result of ~30 minutes
- Quickly adaptable if the virus mutates over time or if new viruses emerge that have potential to cause a pandemic
- FDA IVD clearance and CE-IVD mark
- CLIA-waived for point of care use
- “High” sensitivity and specificity

Simplifying the GeneXpert Platform

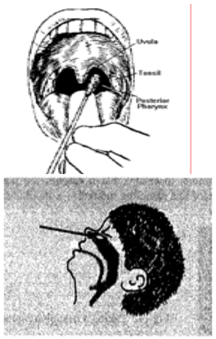
- GeneXpert I
  - Windows OS
  - Standard GX Software
  - Touch Screen on External Laptop
- Portable
  - Only 14 pounds

Xpert Flu Design Implementation

Attribute	Solution
Assay Format	Single cartridge detection of Flu A, Flu B, H1, H3, H5, and a sample preparation/internal control ● Six-color real time fluorescence
Time to Result of ~30 Minutes	Sample prep to be completed in approximately 10 minutes. Fast RT-PCR to be completed in approximately 20 minutes ● One-step RT-PCR assay
Sample Types	Nasalpharyngeal and/or throat swab into viral transport medium or directly into cartridge
Instrument for POC	Single module GeneXpert instrument ● Touch screen on external laptop

Xpert Flu Assay Protocol

Collection device
Swab + sponge soaked with VTM



Throat swab and/or nasal swab

Place swab(s) into cartridge

Break swab(s) into chamber, close lid

Scan cartridge barcode load into GX

Lyse
Cells and Virus

Bind + Wash
Nucleic Acids

Elute
Nucleic acid

Resuspend
Flu A/Flu B/H1/H3/SPC TSR bead
H5 TSR bead
RT-PCR EZR bead

One Step RT-PCR

Flu A	Flu B	H1	H3	H5	SPC/IC	Test Result
NEG	NEG	NEG	NEG	NEG	POS	Flu not detected
NEG	NEG	NEG	NEG	NEG	NEG	Invalid
POS	NEG	NEG	NEG	NEG	POS	Flu A detected
POS	NEG	NEG	NEG	NEG	NEG	Flu A detected
POS	NEG	POS	NEG	NEG	POS	Flu A H1 detected
POS	NEG	POS	NEG	NEG	NEG	Flu A H1 detected
POS	NEG	NEG	POS	NEG	POS	Flu A H3 detected
POS	NEG	NEG	POS	NEG	NEG	Flu A H3 detected
POS	NEG	NEG	NEG	POS	POS	Flu A H5 detected
POS	NEG	NEG	NEG	POS	NEG	Flu A H5 detected
NEG	POS	NEG	NEG	NEG	POS	Flu B detected
NEG	POS	NEG	NEG	NEG	NEG	Flu B detected
POS	POS	NEG	NEG	NEG	POS	Flu A and Flu B detected
POS	POS	NEG	NEG	NEG	NEG	Flu A and Flu B detected
POS	POS	POS	NEG	NEG	POS	Flu A H1 and Flu B detected
POS	POS	POS	NEG	NEG	NEG	Flu A H1 and Flu B detected
POS	POS	NEG	POS	NEG	POS	Flu A H3 and Flu B detected
POS	POS	NEG	POS	NEG	NEG	Flu A H3 and Flu B detected
POS	POS	NEG	NEG	POS	POS	Flu A H5 and Flu B detected
POS	POS	NEG	NEG	POS	NEG	Flu A H5 and Flu B detected

Cepheid Sample in. Answer out.™

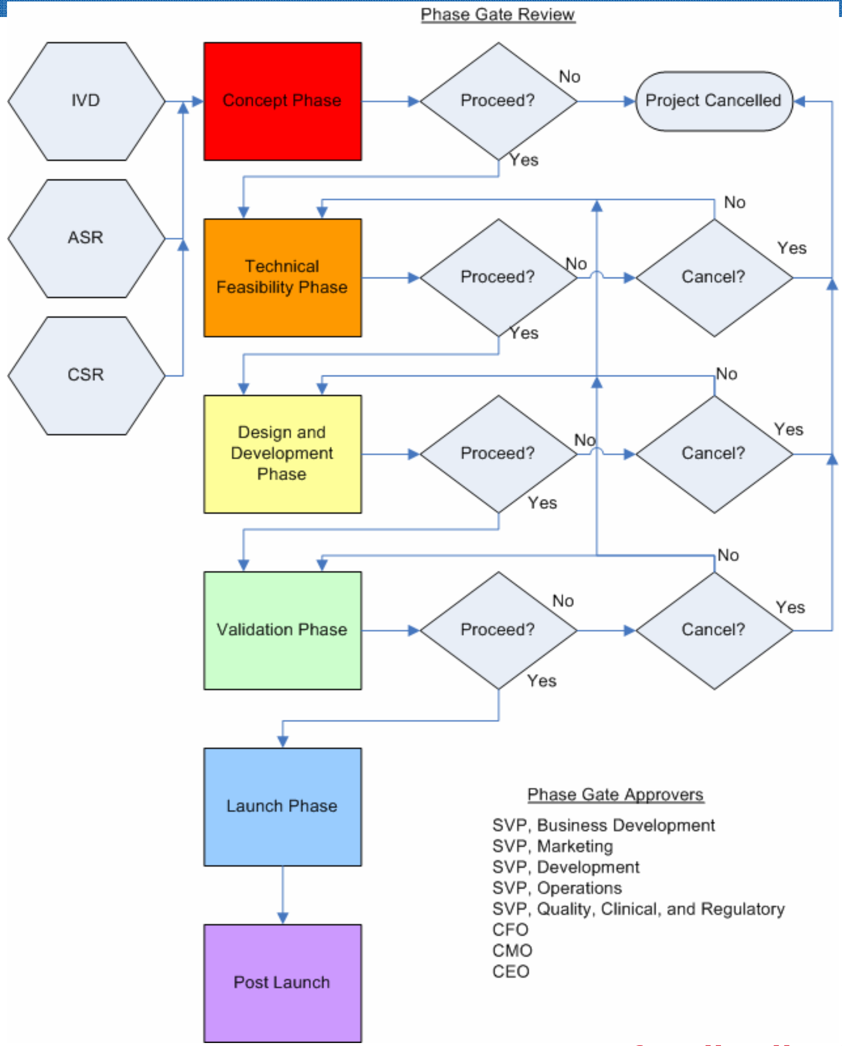
Amplification Protocols

- Multiple stages cycling protocols
- Advance-to-next-stage (ANS)
  - Enables temperature-limited priming or temp-controlled amplicon melting
- Bifurcated advance-to-next stage (BANS)
  - Enables combination of 2 or more quenching strategies
- Sequential reaction protocols
  - Enables 2-step RT-PCR; nested PCR; multiple nested PCRs after initial PCR, WGA, or 1-step RT-PCR

Chemistry Initiatives

- Establish a organic chemistry capability
  - Novel fluorescent dyes
    - Needed for advanced, high multiplexing in solution phase reactions (6-color)
  - Novel, more efficient and cost-effective quenchers
  - Linkage chemistry for easier dual-labeled oligos (probes)
  - Modified bases
- Establish oligonucleotide synthesis capability
  - Develop improved methods for cost-effective
  - Rapid turnaround of development materials
  - Enable incorporation of novel, modified bases and nucleotides to enhance assay performance
    - Improved base match/mismatch discrimination
    - Higher primer and probe Tms for increase speed of PCR reaction
    - Simplify assay design for targets with high polymorphisms
    - Reduce non-specific interactions between primers and probes in highly multiplexed reactions

Regulatory: Design Control



Alpha testing

Beta testing

Clinical trials

GeneXpert Production

Sample in. Answer out.™

Operations Overview

Global Operations

- Corporate Offices
- Assay R&D
- Systems R&D
- Manufacturing

- Chemistry & Oligo R&D
- Chemistry & Oligo Mfg.

- Sales & Marketing
- micro RNA Research

- Assay R&D
- Manufacturing
- Microbiology Testing

MANUFACTURING

GeneXpert®

Cepheid

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Manufacturing

GeneXpert®

I-CORE®

X 1

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Manufacturing

GeneXpert®

X 1

X 2

X 3

X 4

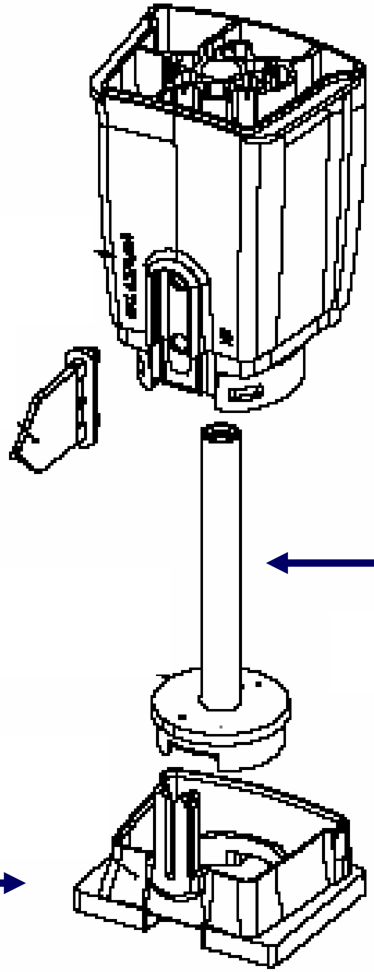
X16

Flexible Configurations

Cepheid Sample in. Answer out.™

Manufacturing

GeneXpert® Cartridge Assembly



Cartridge Body
- 11 Chambers
  - Reagents
  - Buffer
  - Sample
  - Waste

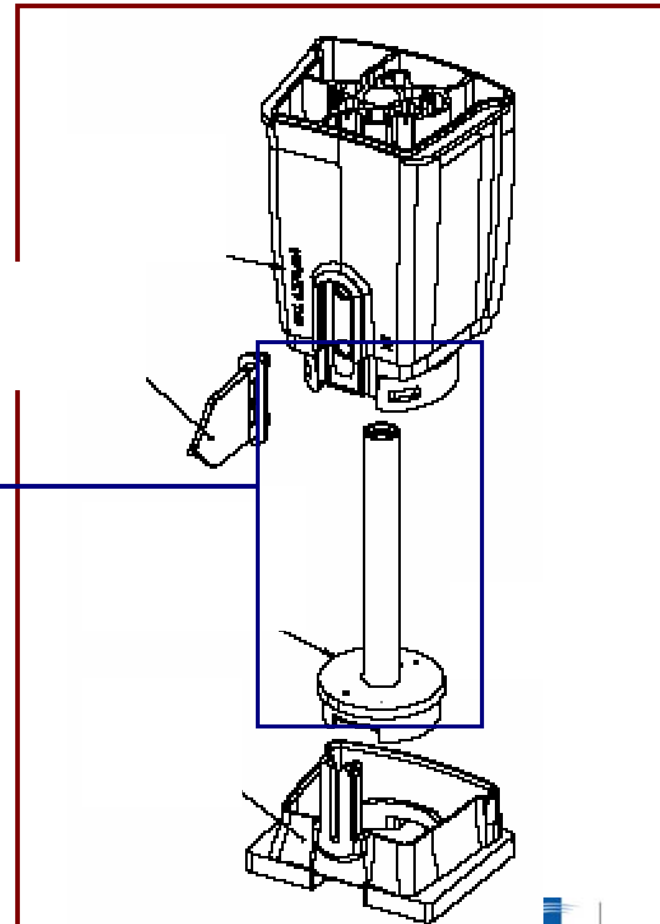
Cartridge Tube

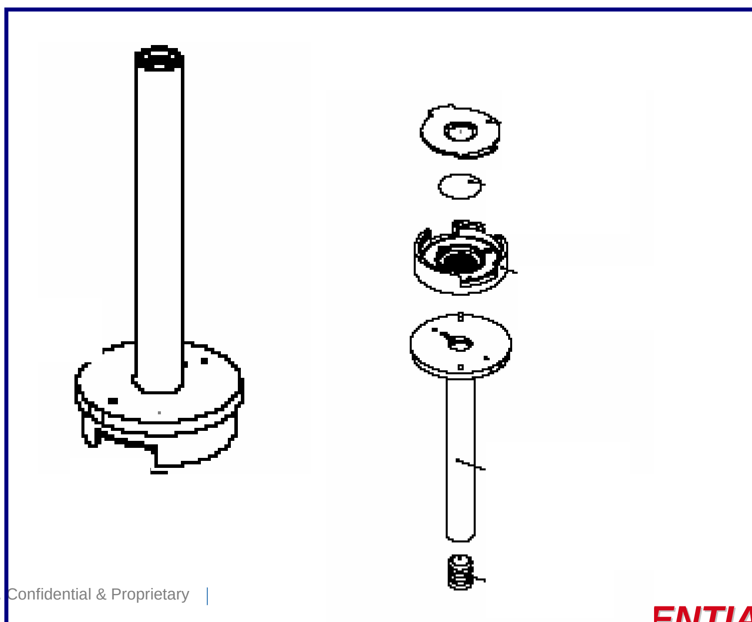
Valve Body
- Syringe Drive
  - Plunger
  - Glass Beads
  - Filter

Foot

Manufacturing

GeneXpert® Cartridge Valve Body





Manufacturing

GeneXpert® Cartridge Assembly

Overall View

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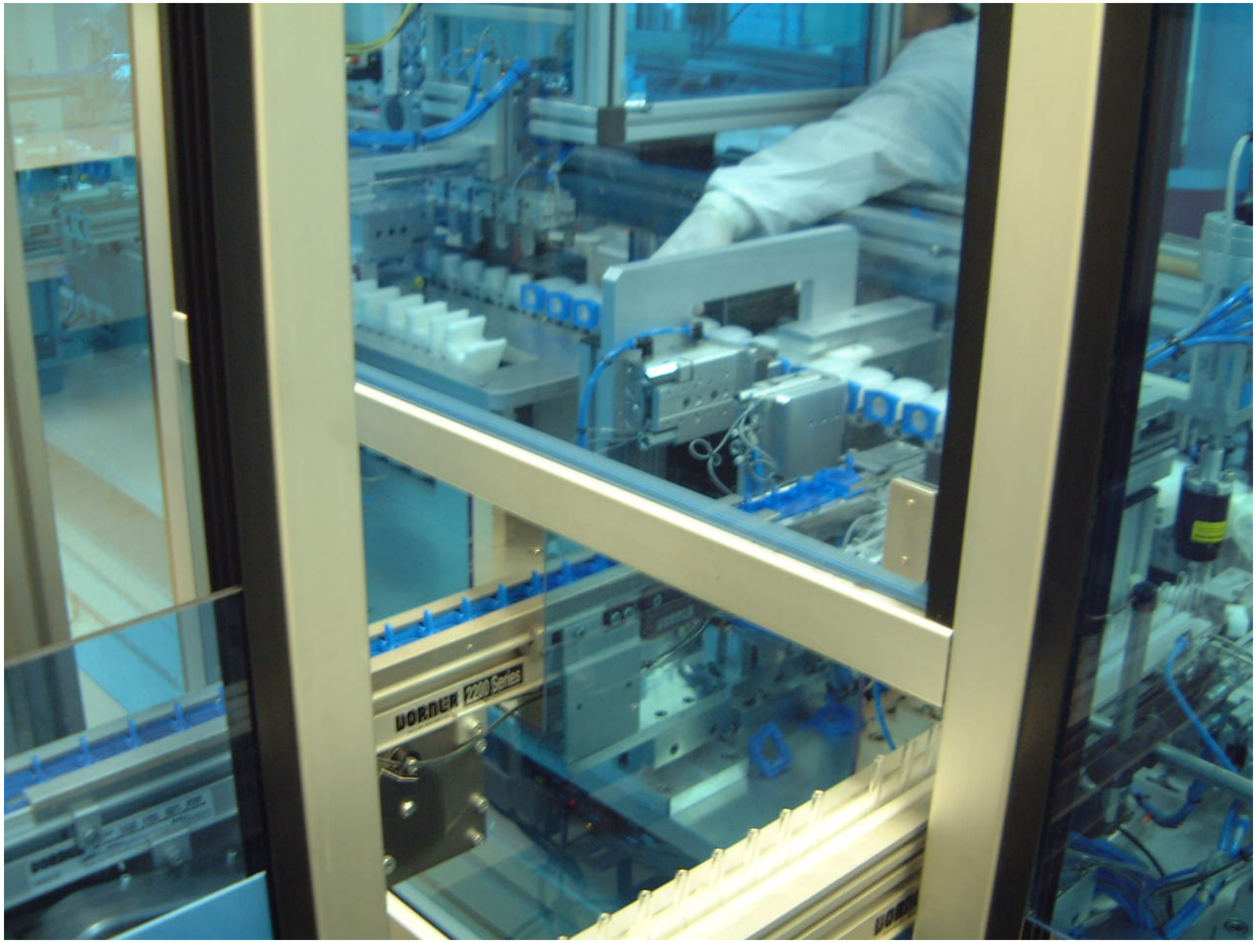
Manufacturing

GeneXpert® Cartridge Assembly

Body & Tube On-load

Manufacturing

GeneXpert® Cartridge Assembly



Valve & Foot On-load

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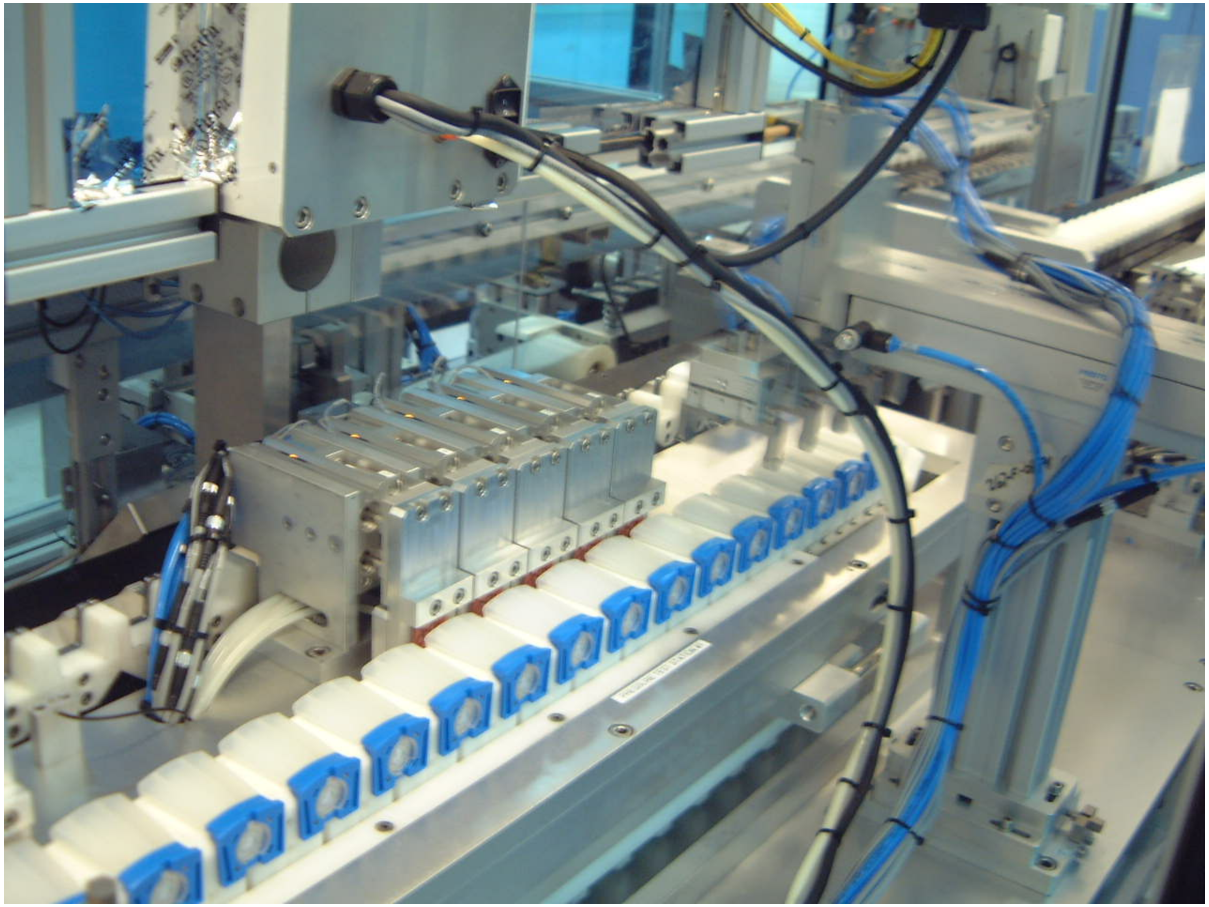
Manufacturing

GeneXpert® Cartridge Assembly



Assembly & Test

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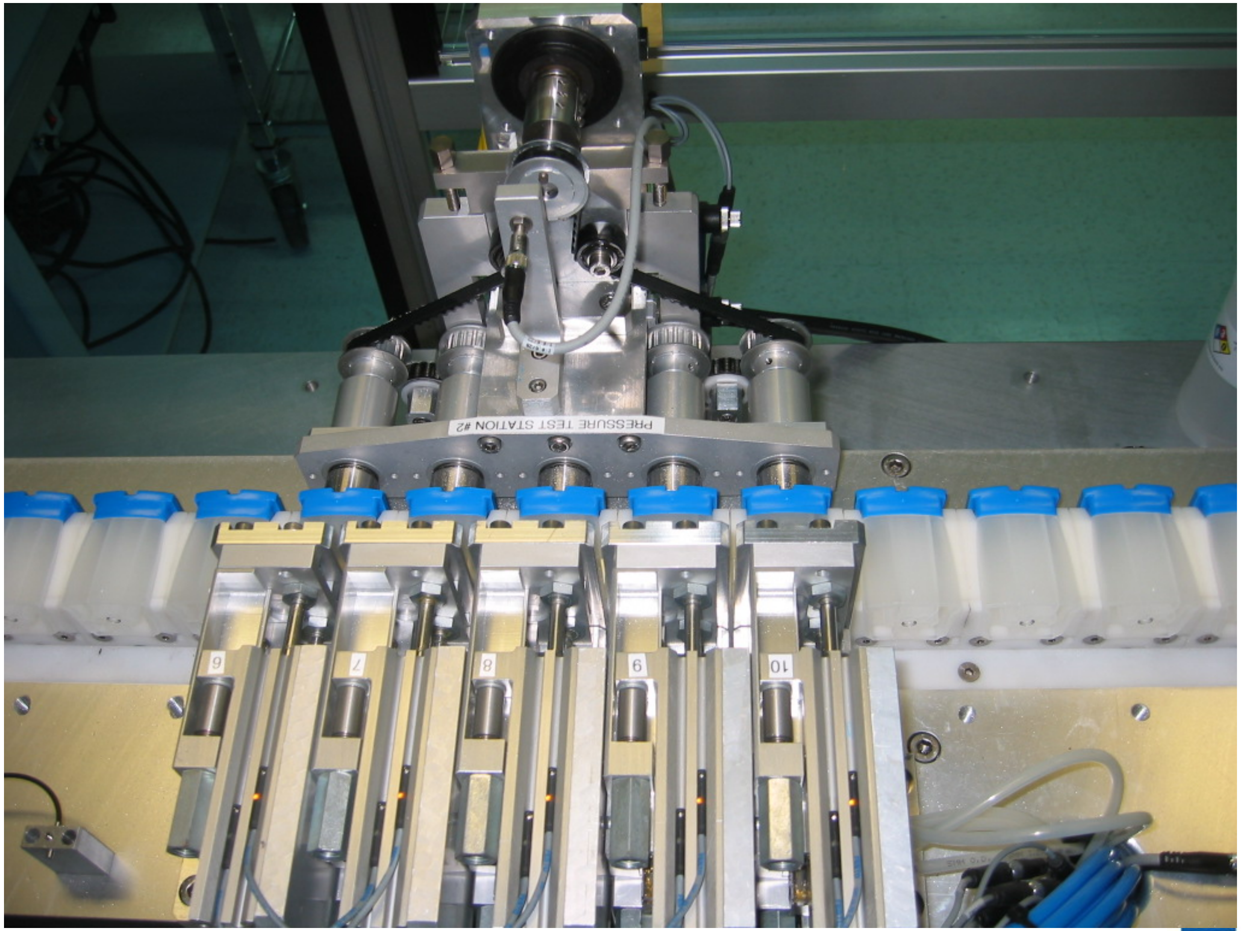
Manufacturing

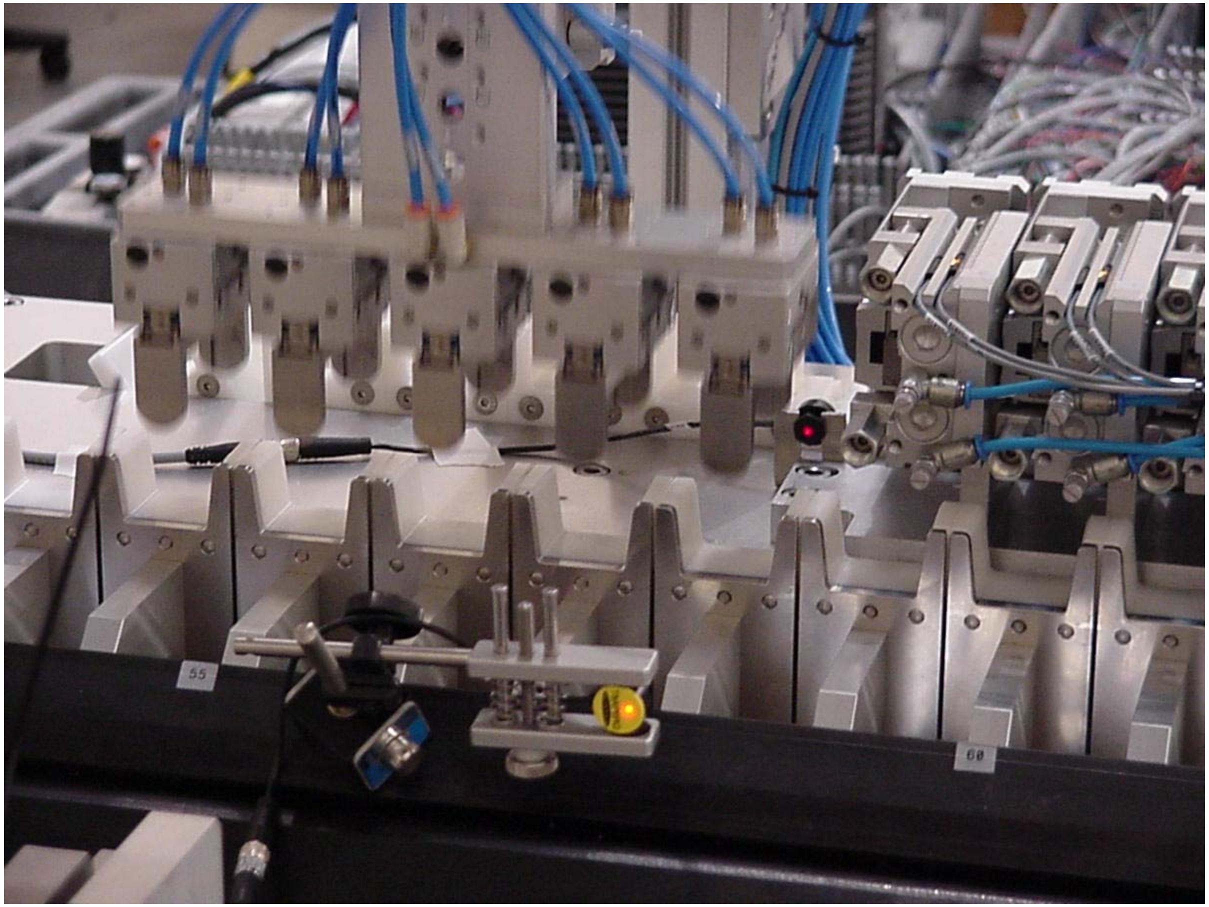
GeneXpert® Cartridge Assembly



Test Rotation

Manufacturing

GeneXpert® Cartridge Assembly



Transfer

Cepheid Sample in. Answer out.™

Manufacturing

GeneXpert® Cartridge Assembly

Bead Fill

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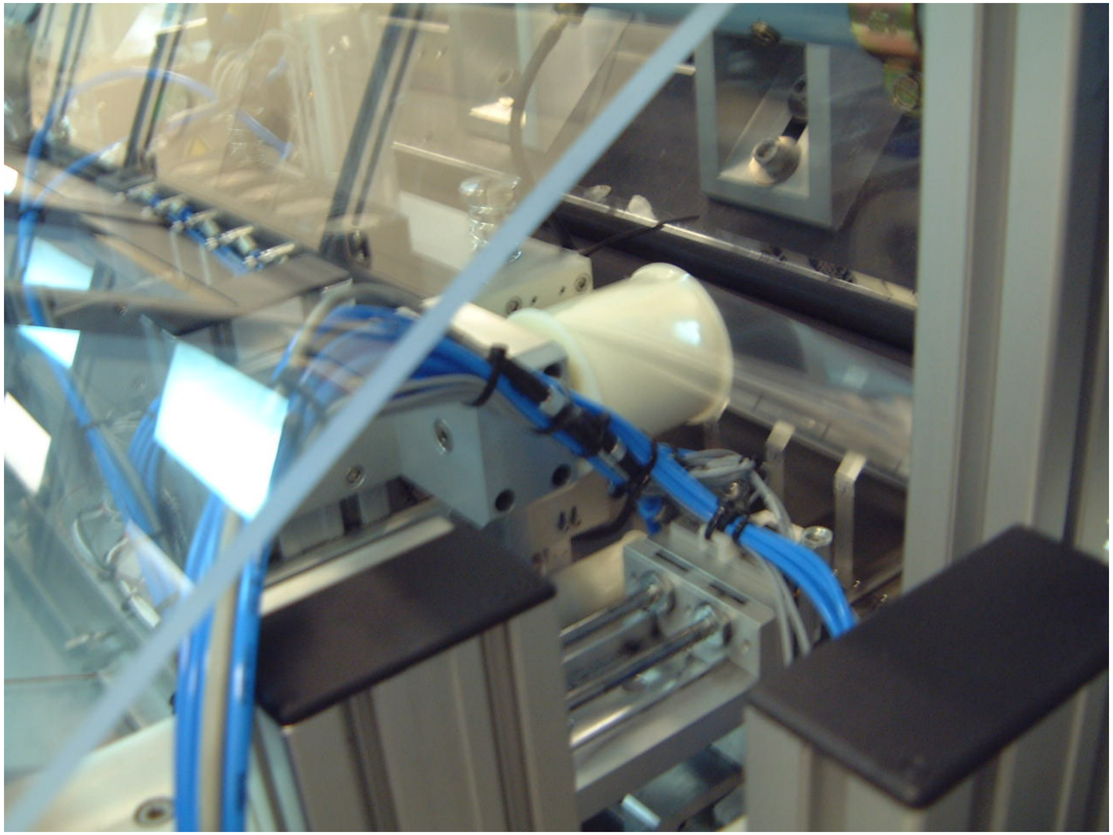
Cepheid

Sample in. Answer out.™

MANUFACTURING

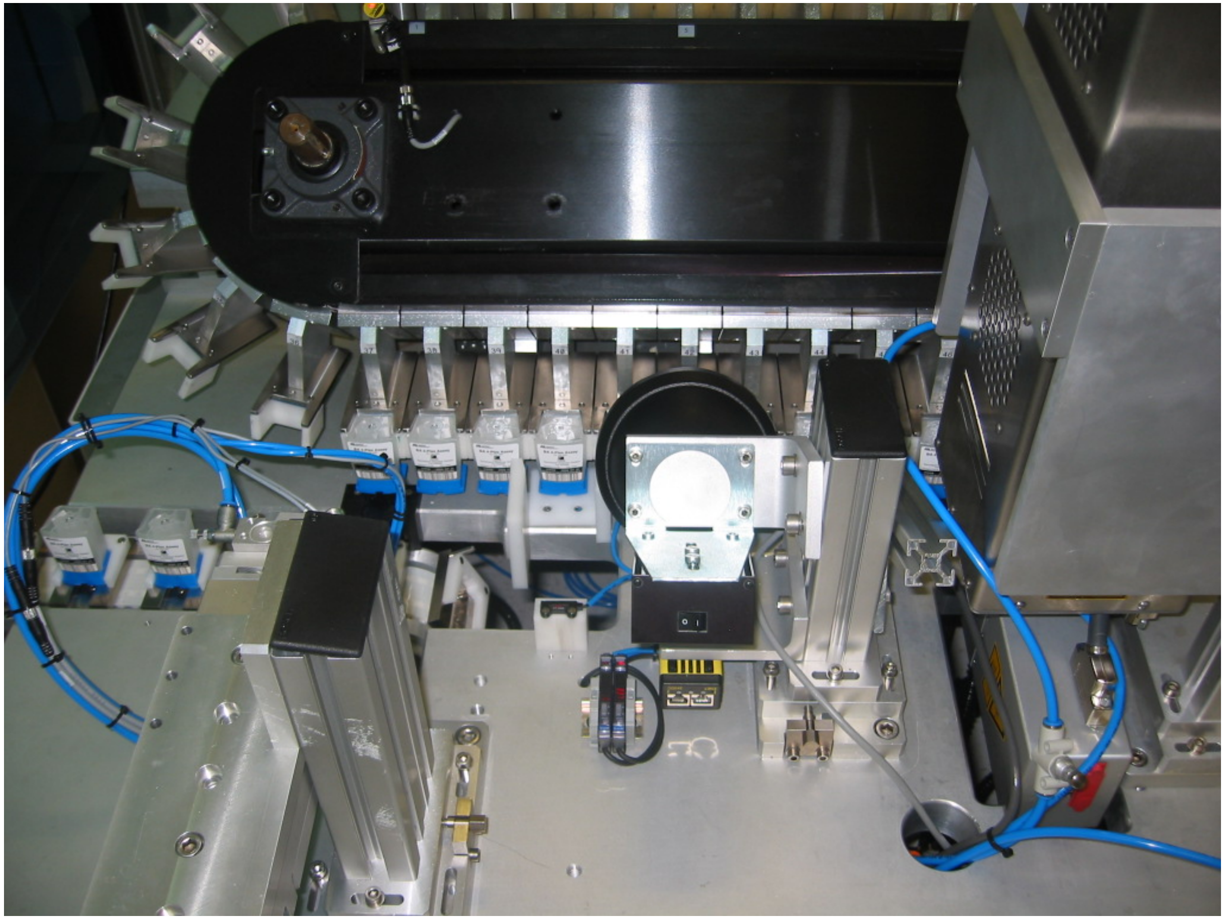
GeneXpert® Cartridge Assembly



Seal

Manufacturing

GeneXpert® Cartridge Assembly

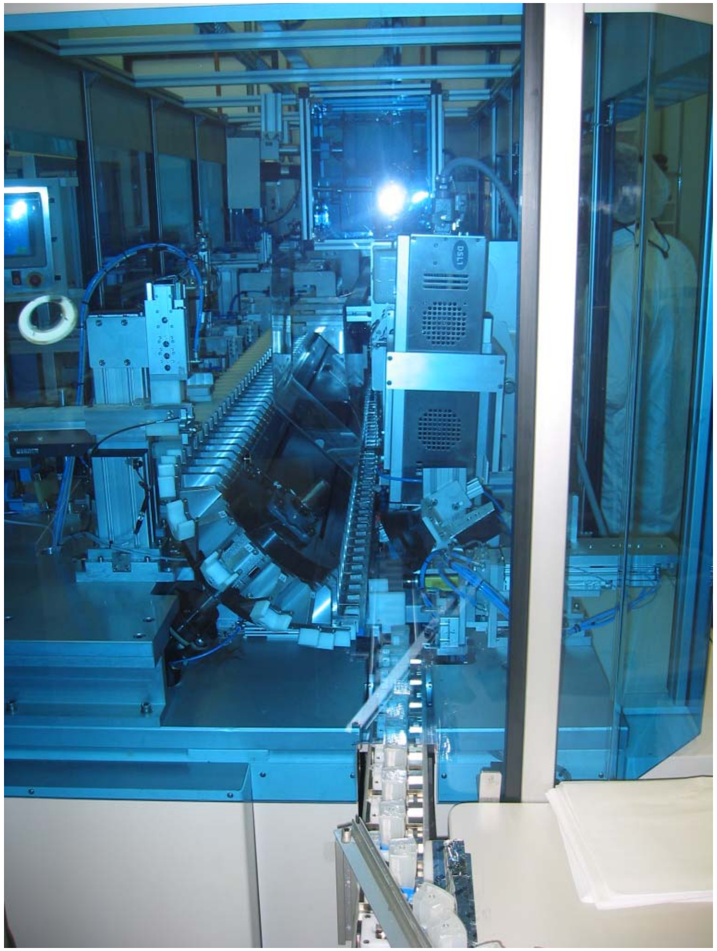


Label Application

Cepheid Sample in. Answer out.™

Manufacturing

GeneXpert® Cartridge Assembly



Off-load

Cepheid

Sample in. Answer out.™

Manufacturing

GeneXpert® Cartridge Assembly

Finished Part

Algorithms, Assays And Samples?

ILI/URI.1
Influenza A
Influenza B
Adenovirus - pan
Adenovirus - 4
Respiratory syncytial virus
Rhinovirus*
Human parainfluenza 3
Mycoplasma pneumoniae
Bordetella pertussis .1
Bordetella pertussis .2
Streptococcus pyogenes

ILI/URI.2
Human metapneumovirus
Human parainfluenza virus 1
Human parainfluenza virus 2
Human parainfluenza virus 4*
Enterovirus*
Human coronavirus 229E*
Human coronavirus OC43*
Human coronavirus NL63*
Human coronavirus HKU1*
Chlamydia pneumoniae

Flu - typing
Influenza A
H1
H3
H5A
H5B
H7
H9
Influenza B

Pneumonia
Influenza A
Adenovirus - pan
Streptococcus pneumoniae
Chlamydia pneumoniae
Mycoplasma pneumoniae
Legionella "species"
*Haemophilus influenzae***
Streptococcus pyogenes
Staphylococcus aureus
Neisseria meningitidis "Y"**
*Moraxella catarrhalis***

ILI consensus
Influenza A
Influenza B
Adenovirus - pan
Respiratory syncytial virus
Human parainfluenza 3
Mycoplasma pneumoniae
Bordetella pertussis .1
Bordetella pertussis .2
Streptococcus pyogenes
Chlamydia pneumoniae

Algorithms, Assays And Samples?

ILI/URI.1
Influenza A X
Influenza B X
Adenovirus - pan
Adenovirus - 4
Respiratory syncytial virus* XX
Rhinovirus*
Human parainfluenza 3
Mycoplasma pneumoniae XX
Bordetella pertussis .1 XX
Bordetella pertussis .2 XX
Streptococcus pyogenes

ILI/URI.2
Human metapneumovirus
Human parainfluenza virus 1
Human parainfluenza virus 2
Human parainfluenza virus 4*
Enterovirus* X
Human coronavirus 229E*
Human coronavirus OC43*
Human coronavirus NL63*
Human coronavirus HKU1*
Chlamydia pneumoniae XX

Flu - typing
Influenza A X
H1 X
H3 X
H5A X
H5B
H7
H9
Influenza B X

Pneumonia
Influenza A X
Adenovirus - pan
Streptococcus pneumoniae
Chlamydia pneumoniae XX
Mycoplasma pneumoniae XX
Legionella "species"
*Haemophilus influenzae***
Streptococcus pyogenes
Staphylococcus aureus X
Neisseria meningitidis "Y"**
*Moraxella catarrhalis***

ILI consensus
Influenza A X
Influenza B X
Adenovirus - pan
Respiratory syncytial virus XX
Human parainfluenza 3
Mycoplasma pneumoniae XX
Bordetella pertussis .1 XX
Bordetella pertussis .2XX
Streptococcus pyogenes
Chlamydia pneumoniae XX

GX Product/ in Development X

ASR/Cepheid AB Product/ in Development XX

Cepheid | Sample in. Answer out.™

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Proposal Detection of Respiratory Viruses and other Infectious Disease Agents with an Automated System	
Offeror Nanogen, Inc.	*Offeror type of Organization* Publicly Traded, For Profit United States-based
Technical and Project Contact William G. Weisburg, Ph.D. 10398 Pacific Center Court San Diego, CA 92121 Tel: 858.410.4646 Fax: 858.410.4952 wweisburg@nanogen.com	*Business and Corporate Contact* Graham P. Lidgard, Ph.D. 10398 Pacific Center Court San Diego, CA 92121 Tel: 858.410.4794 Fax: 858.410.4848 glidgard@nanogen.com
Date Proposal Submitted July 13, 2007	
Proposed Project Start Date October 1, 2007	*Offeror's Proposal Expiration Date* March 31, 2008
Requested Funding $19,750,000	*Project Duration* October 1, 2007 – September 30, 2011 (alternatively, four years from project start date)
Funding Allocation for Requested Funding Project Year 1: $1,850,000 Project Year 2: $3,900,000 Project Year 3: $7,000,000 Project Year 4: $7,000,000	*Offeror's Estimated Additional Contribution to Funding* $20,250,000 Nanogen contribution (includes about $5,000,000 of anticipated funds from grants)

Brief Description of Proposed Program

The communicable nature of infectious diseases creates both an immediate issue of managing the patient and a broader issue of quelling the spread of infection. For patient management, selection of an appropriate antibiotic or antiviral will reduce morbidity and mortality. In institutional settings, such as schools, hospitals, military bases, and ships, it is important to rapidly identify the presence of an infection and manage the potential for further infections. This proposal addresses the need for rapid diagnostic methods to enable the management of patients and enable the suppression of potential outbreaks. The offeror proposes to develop an automated system for the detection of specific etiological agents causing epidemic infectious diseases by employing molecular diagnostic methods. Specifically, Nanogen will use their established sample preparation, DNA amplification, and electronic microarray detection to determine which, if any, of a set of pathogenic agents of disease is present in a clinical sample. The initial assay proposed will screen for any of a set of eight viral respiratory agents of significance to the Department of Defense. This panel will be followed by a test for atypical pneumonia bacteria, and then followed by a panel of meningitis causing agents. The automated system will be designed for minimal technologist skill, low maintenance, small footprint, and robust electromechanical components. The proposed system is intended to be suitable for use on ships and in similar limited space situations. It is intended that the respiratory viral panel should be cleared by the FDA by the end of the project and the atypical pneumonia and meningitis assays are ready to initiate clinical trials. In anticipation that the resulting assays and the instrument platform will have high value for military and civilian diagnostic settings, the offeror is also contributing their own and other grant funds to this valuable program.

White Paper / Proposal

Detection of Respiratory Viruses and other Infectious Disease Agents with an Automated System

1. Introduction

The diagnosis and identification of infectious diseases is done by a composite of diverse primary methods. The infectious agents causing these diseases of various organ systems may be bacterial, viral, fungal, or parasitic in nature. Methods used for detection include culture, serology, antigen detection, direct microscopic examination, or molecular diagnostics. Among those methods, molecular diagnostic technology has seen the greatest recent growth, attributable to several benefits of molecular technology: (1) high sensitivity and specificity, (2) suitability for detecting agents that are difficult to culture such as viruses and fastidious bacteria, (3) stable specimen transport compared to culture, (4) neutrality of the technology to the taxonomy of the agent (technology for detecting viruses is the same as that used from bacteria or fungi), and (5) suitability for automation.

Nucleic acid detection methods are the standard of care for sexually-transmitted diseases, human papilloma virus detection, and quantitation of HIV-1 viral load, to cite just a few examples. While the technology has been widely adopted for detecting one or sometimes two pathogens in a sample, molecular diagnostic technology has not been generally available for the detection of groups of pathogens. For example, respiratory illness can be caused by any among several viruses and bacteria. The existing molecular technology would look for one pathogen at a time, whereas viral culture is inherently multiplexed. This proposal is focused on the next generation of molecular diagnostic technology, testing for a panel of pathogens from a single sample in order to identify rapidly what agent is causing disease.

Unsuitability of molecular diagnostic technology for panels is only one of the deficiencies of extant diagnostic products. Complexity, expensive and large instrumentation, and high skill level define the other critical shortcoming of molecular diagnostic testing. Dedicated technologists and large robotic platforms may be suitable for very high throughput batch testing in centralized laboratories, but unsuitable for smaller labs, where diagnostic results are required in a few hours.

Nanogen entered the molecular diagnostic arena through pioneering development of an electronic microarray. The hallmark of this technology is the ability to greatly accelerate the kinetics of hybridization to DNA probe arrays (chips, biochips) by employing electronic field concentration. DNA hybridization events that take ten or more hours on conventional biochips can be completed in two minutes on an electronic microarray.

Recently, Nanogen has focused research effort on the processes of sample purification, reverse transcription (RT) and polymerase chain reaction (PCR, or combined with reverse transcription, RT-PCR) as part of a total assay process with the microarray. With these three fundamental processes—sample preparation, RT-PCR, and array-based detection—Nanogen has a technological foundation for vastly improved testing of panels of infectious agents. Nanogen's objective, which is consistent with the mission and needs of the United States Department of Defense, is to automate molecular diagnostic panel testing for smaller laboratories.

Nanogen proposes herein to develop a system with the following attributes:

1. Walk away molecular diagnostic platform
2. Initial panel for the detection of viruses causing significant respiratory illness
3. Capable of operation and routine maintenance by technicians of intermediate skill and training
4. Time to result of three hours or less (with a project target of less than 90 minutes)
5. Small footprint within a mechanically robust system suitable for placement on ships
6. FDA clearance of the initial viral detection panel
7. Menu expansion for further panels for other disease states such as atypical pneumonia, meningitis, and diarrhea-causing pathogens

Infectious diseases, especially respiratory viral infections, are highly transmissible. It is likely that an infected individual, once he or she is diagnosed by conventional means, will have infected several other persons; thus, the occurrence of a growing dendrite of infection. Institutional settings, with close proximity of potentially infected people, are particular hot spots for outbreaks. Controlling the spread of respiratory viral infection, through the implementation of more rapid diagnostic methods, is a medically and economically significant goal.

There is some debate about the precise composition of such a panel and about the addition of certain taxa, such as coronavirus. The consensus opinion is that this list captures the most important viruses involved in community-acquired respiratory infections:

1. Influenza A (fluA)
2. Influenza B (fluB)
3. Respiratory syncytial virus A/B (RSV)
4. Human parainfluenza virus 1 (HPIV-1)
5. Human parainfluenza virus 2 (HPIV-2)
6. Human parainfluenza virus 3 (HPIV-3)
7. Human metapneumovirus (hMPV)
8. Adenovirus (minimally, subspecies B, C, and E [serotype 4])

Conventional means for detection of these viral infections include viral culture, direct fluorescent antibody staining, rapid lateral flow assays (for some of these), and in some laboratories, real time PCR. Most laboratories do not currently perform testing for human metapneumovirus, while acknowledging that hMPV is a significant agent of respiratory disease.

When the proposed program is successfully completed, it will be possible for a technologist to take a patient's swab sample and run a fully automated multiplex PCR assay by simply adding the sample to a disposable device, putting the disposable in an instrument, pushing a few buttons (such as a touch screen), and coming back in approximately 2 to 3 hours (target for project will be less than 90 minutes) for results. This will enable far more timely management of the patient and equip the appropriate institution to manage and potentially avoid an epidemic outbreak.

Respiratory viruses are just the first of a potential menu of infectious disease panels for the new platform that will be developed in this program. These viruses will be followed by additional panels: (1) bacterial atypical pneumonia agents, (2) meningitis and encephalitis pathogens, (3) agents of diarrheal disease, and potentially (4) a biothreat agent panel.

2. Specific Aims

The overall aim of the proposed project is to develop an automated system for the laboratory that will process clinical samples and yield diagnoses for a panel of infectious disease agents, specifically, respiratory viruses in the first panel. This will be accomplished through the achievement of a series of specific aims, which can be further understood through the Proposed Effort, below.

<u>Specific Aim 1: Phase I Feasibility:</u> Completion of a feasibility milestone for a respiratory viral panel on an open system which includes sample preparation, a block thermocycler, and the NanoChip 400 system for detection. In this phase, the final list of etiological agents will be identified, and bioinformatics analysis will be completed. Testing will yield primers, capture probes, and reporter probes with requisite inclusivity and exclusivity. Multiplex PCR amplification reactions will be optimized and multiplex detection will be developed on the NanoChip 400. A pilot clinical study will demonstrate the clinical utility of the assay. This Specific Aim will yield the basic chemistry of the assay that will then be automated (with predictable iterations) and serve as the benchmark for all testing of the automation and integration.

Specific Aim 1 will be completed 12 months after the start of the project

<u>Specific Aim 2: Modules/Test Fixtures:</u> Fundamentally, the system executes three separate processes: sample preparation/purification, PCR amplification, and electronic microarray detection.

The initial approach to automation will seek to take these three processes, as practiced in conventional laboratory systems, and render each one practicable in a dedicated test fixture. It is expected that this stage of the program will be iterative and that some changes will be made to the chemistry to suit the materials, fluid dynamics, and other unique aspects of a closed fixture.

Specific Aim 2 will be completed 15 months after the start of the project

Specific Aim 3: Breadboard: A key milestone in the program will be this breadboard phase. The three separate functions of sample preparation, amplification, and detection will be brought together (albeit possibly crudely) in a manner that now allows processing of samples through an entire assay. Throughput will likely be lower and failure rates higher than a final system, but it will be possible, at this stage, to run enough samples through the respiratory viral panel assay to determine the viability of the system as a whole.

Specific Aim 3 will be completed 21 months after the start of the project

Specific Aim 4: Integrated Alpha System: Integration and development of an alpha system will primarily be noted by the development of a single disposable unit, which is in its final configuration, and the instrument that runs the disposable will be in a penultimate configuration. At this stage, planning for production capacity are a high priority, in order to ensure that this can be a viable, cost-effective product.

Specific Aim 4 will be completed 27 months after the start of the project

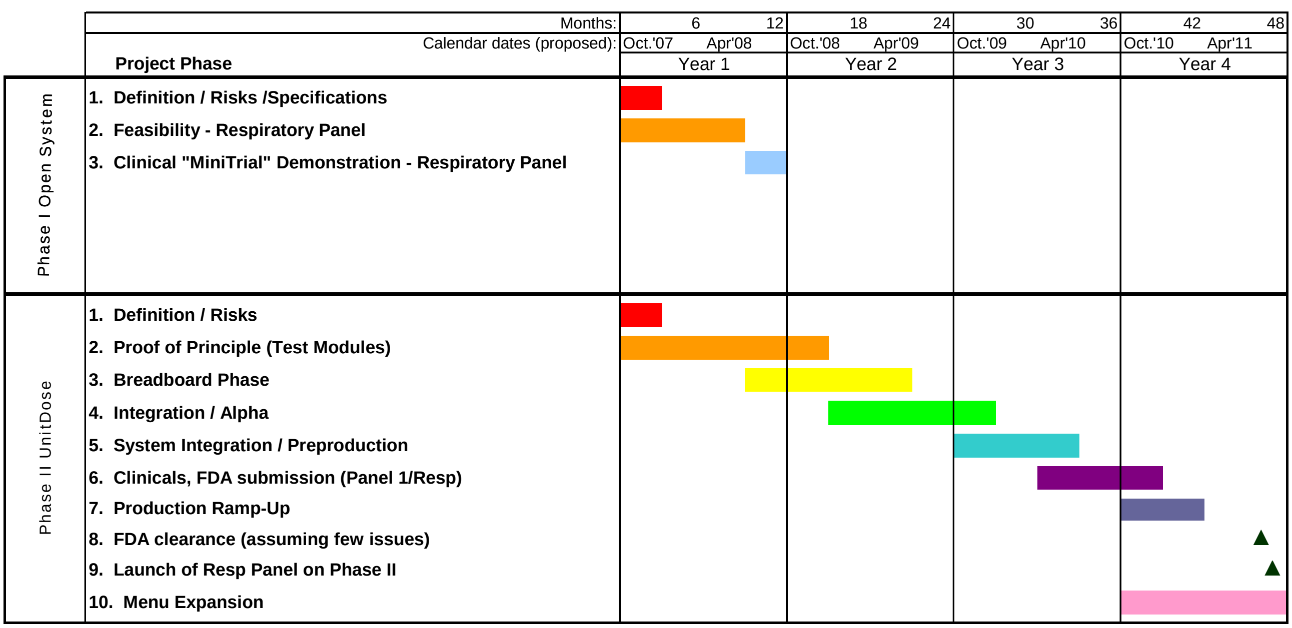
Specific Aim 5: Clinical Trials/Submission: A pivotal aspect of the program is the execution of clinical trials followed by submission of the data, and all other necessary labeling and supporting information, to the Office of In Vitro Devices at FDA/CDRH.

Specific Aim 5 will be completed 39 months after the start of the project

Specific Aim 6: Approval and Launch: Approval and launch will then signal the end to this particular phase of the program.

Specific Aim 6 will be completed 48 months after the start of the project

Schedule of Specific Aims



3. Technical Background

Electronic microarray technology: Nanogen has engaged in the development of electronic microarray technology for more than ten years. The technology combines electrophoretic movement of biomolecules, microelectronics, DNA hybridization, and fluorescent detection. The hallmark differences between this technology and other microarray technology is threefold:

1. Electronic microarray need not be “pre-printed.” Building an array can be part of the process of running the assay, allowing for greater flexibility. If it is desirable to have a pre-printed array, the Nanogen technology can be employed in this manner.
2. The solution added to the chamber of the electronic array, when selected array addresses are activated…
3. The kinetics of hybridization are slow when binding DNA to complementary sequence on conventional two-dimensional arrays. The kinetics are first order, driven by the concentration of the DNA in solution. Consequently, hybridization of PCR amplicon mixes to arrays frequently requires several hours—overnight is not unusual. The electronic microarray, by activating a small feature on the surface of the array with a positive charge, concentrates the DNA at that feature and drives the hybridization reaction, typically with a two minute duration.

The electronic microarray technology is covered by about one hundred issued United States patents.

The NanoChip 400: The NanoChip 400 (NC400) is the current platform from Nanogen for running electronic microarray assay. The instrument is fully automated and performs the following functions:

1. Electronic addressing of capture probes to specific locations on the pad to prepare an array appropriate for the assay that is being run
2. Robotic sampling from a sealed PCR reaction plate of a multiplex PCR reaction
3. Injection of the sample into the sample chamber over the array
4. Electronic addressing of the sample to a set of capture probe laden pads for each sample
5. Washing out excess sample from the sample chamber
6. Addressing of the next sample as in step 4 and 5, above, through the completion of applying each sample for analysis
7. Hybridization of reporter probes (discriminators)
8. Washing out excess reporter probe
9. Fluorescent read of the array and data capture
10. Result interpretation

Fig 1. Rendering of a captured target complex on a NC400 pad

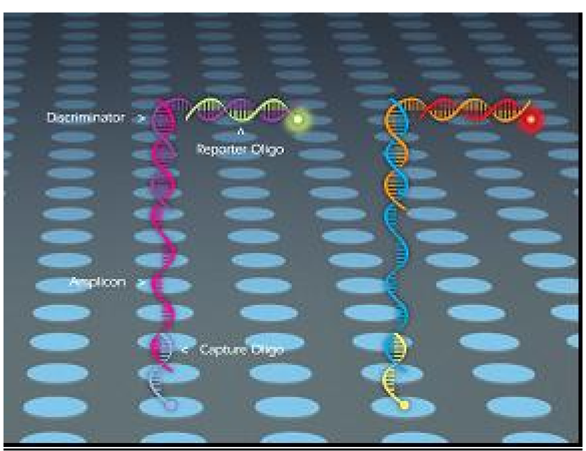


Fig 2. The NanoChip 400 Instrument

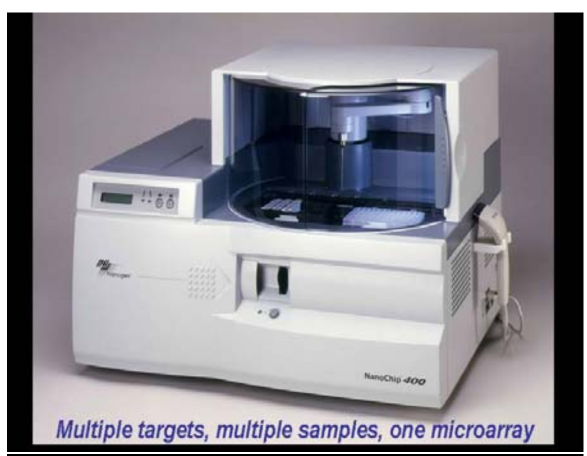


Figure 1, above, shows a depiction of the surface of the array with a PCR amplified target bound to a capture probe and a fluorescent-labeled reporter probe.

Figure 2 shows the NanoChip 400 instrument.

The utility of electronic microarrays is unique compared to other microarray methods in two primary ways:

(1) A single microarray can be used for the detection and identification of numerous samples, for example 100 samples could be tested across four pads, for a total of eight analytes per sample (using two fluorescent dyes) on a 400 site cartridge. This is accomplished because the specificity of activating sets of individual capture sites prevents cross talk of different samples.

(2) The time to result of an assay is far lower, due to the technology advantages cited above.

Commercial applications have been developed, including:

1. Analyte Specific Reagents for the detection of respiratory viruses (see below)
2. Analyte Specific Reagents for the detection of 23 significant mutations of the CFTR (or Cystic Fibrosis Transmembrane Conductance Regulator) gene
3. Research Use Only reagents for the detection of variants of the cytochrome P450 CYP2C9 locus
4. *In vitro* diagnostic kits for the detection of 23 key mutations of the CFTR gene have been submitted for pre-market evaluation to the U.S. FDA and awaiting 510(k) clearance
5. Other genetic tests are in the final stages of development, including reagents for detection of mutations of Factor V (Leiden) and prothrombin.

Additional array-based assays for detection or identification of infectious diseases have been developed. A grant from the National Institute of Allergy and Infectious Diseases supports a project titled *Diagnostics for Sepsis and Community Acquired Pneumonia*. The principal investigator is William Weisburg, Ph.D. of Nanogen, and the Medical College of Wisconsin (MCW) is a subcontractor through Kelly Henrickson, M.D. The grant number is 5U01 AI066584, and spans July 2005 to June 2010. The assay has been designed and optimized to detect the following taxa and loci on the NanoChip 400 in a multiplex format:

Influenza A	Segment 7: Matrix Protein M1	233 bp
Influenza B	Non-Structural Protein NS1/NS2	244 bp
Respiratory Syncytial Virus A/B	N/NS2 Spacer and Gene	149/209 bp
Mycoplasma pneumoniae	P1 Cytadhesin Gene	300 bp
Chlamydophila pneumoniae	MOMP Gene	232 bp
Legionella pneumophila	Macrophage Inf. Potentiator	160 bp
Legionella micdadei	Macrophage Inf. Potentiator	240 bp
Bordetella pertussis	Porin Gene	132 bp
Streptococcus pneumoniae	Pneumolysin Gene	223 bp
Staphylococcus aureus	Elongation Factor EF-Tu	319 bp

All analytes have achieved a sensitivity of better than 100 copies/mL in a clinical matrix (nasopharyngeal swab in transport media). In addition to the sensitivity study, a carriage study was recently executed (additional data is still being compiled). The data from 100 non-respiratory disease patients' nasopharyngeal swabs are:

Sample Type	Positivity/Negativity	Analyte Found
Nasopharyngeal swab	86%	Negative
	1%	Influenza A (seizure patient)
	9%	*S.pneumoniae*
	3%	*S.aureus*
	1%	*S.aureus* + *S.pneumoniae* (shingles patient)

In addition to the development of the multiplex assay, the grant provides for research towards improvements in sample preparation, amplification, and detection. Significant improvements have been made in all of those areas, for example, increasing the amount of primary sample used in the RT-PCR reaction and decreasing the amount of time necessary for the RT and PCR reactions. The

grant also supports initial experimentation in design of modular test fixtures for each of the three assay steps.

A second grant from NIAID, U01 AI070428, awarded to Kelly Henrickson at MCW, retains Nanogen and William Weisburg, as a subcontractor. Some of the elements of this project are similar to the sepsis and CAP project above. However, this project primarily focuses on bioterrorism and pandemic influenza etiological agents. In accordance with this program, we have recently developed and optimized an assay that determines whether a sample contains sequences for: influenza A, influenza B, influenza A/hemagglutinin H1, H3, or H5, and influenza A/neuraminidase N1 or N2. Thus, one can genotypically identify a sample as containing, for example, influenza A/H3N2, which is fairly common, or A/H5N1 which is a rare avian-borne strain suspected of a potential pandemic threat.

We have demonstrated the ability of this assay to simultaneously type influenza virus and subtype H1N1, H3N2, and H5N1 on an electronic array. Purified RNA transcripts, viral culture material, and clinical samples have all been accurately analyzed. H5N1 isolates from all the major clades were properly typed. Typing was done accurately at concentrations as low as 100 target RNA copies per reaction.

In addition to the assays and the electronic microarray technology, Nanogen has proprietary sample processing chemistry that will be integral to the automation project. We have developed (and applied for a patent) nucleic acid purification chemistry suitable for this project. The performance of this chemistry is very similar to our reference method on the Roche MagNA Pure Compact. In addition, our yields have been experimentally determined as shown in Figure 4.

Fig 3. Nanogen in house proprietary sample prep chemistry

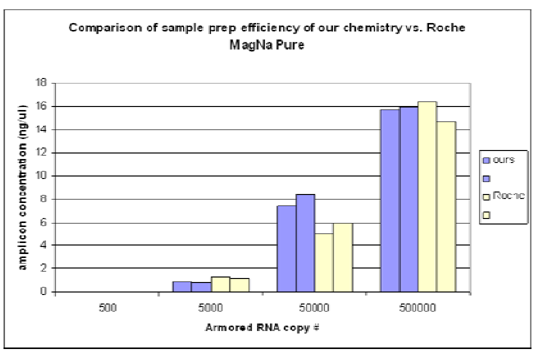


Fig 4. Recovery of nucleic acid yield in Nanogen chemistry

- **Recovery of RNA**
  - **50 - 85% by ^{32}P binding studies**
  - **50 - 70% by amplification assays**
- **Recovery of DNA**
  - **35 - 50% by ^{32}P binding studies**
  - **40 - 50% by amplification assays**

The estimated maximum time budget required, based on experience with the corresponding assay chemistry in a laboratory setting using separate instrumentation, yields two hours thirty minutes time to result. (Project goal is 90 minutes.) The time budget, based on current benchtop systems:

SAMPLE PREP **30 minutes**	**REVERSE TRANSCRIPTION - PCR** **90 minutes** **RT: 20 min / PCR: 70 min**	**ELECTRONIC ARRAY DETECTION** **30 minutes**

4. Description of Proposed System

As described in the Introduction, the proposed project will deliver an automated multiplex molecular diagnostic system for the detection of eight relevant respiratory viral pathogens, with additional infectious disease panels to follow. The target specifications for the system are as follows:

1. Key elements	– Primary sample addition to disposable is only technologist “chemistry” action required – CLIA-waived potential – Panel “syndrome-based” menu with specific analyte result – Detection of RNA and DNA – Mechanically robust, suitable for ship lab space – Room temperature consumable/reagent storage

2.	Sample types	– Swabs: (1) nasopharyngeal, (2) throat, in a transport medium – Cerebrospinal fluid – Perianal swabs if utility warranted
3.	Analytes	– Respiratory viruses – Atypical pneumonia bacteria – Meningitis-causing bacteria – Diarrheal pathogens (TBD)
4.	Sensitivity	– Approximately 100 copy/mL (assuming approx. 0.25 mL primary sample utilization)
5.	Time to result	– ≤ 3 hours (project target will be ≤ 90 minutes)
6.	Target cost of reagents	– ≤ $30 (to customer per panel)
7.	Throughput	– Typical installation (given time to result) yields 20/day in 4 to 5 hours
8.	Target instrument cost	– ≤ $ 6,000 (wholesale)
9.	Quantitation	– Not designed for quantitative assays
10.	Size	– Footprint ≤ 4 sq.ft. (for instrument and operational space) – Weight ≤ 45 lb.

The automated system will execute all of the steps to yield an assay result, starting from the point of the laboratory technologist adding a sample to the system disposable cartridge. It is anticipated that sample addition will employ a measuring syringe (without a needle) or similar easy-to-perform task. The technologist will insert the cartridge into the instrument, record the relevant sample identification data using a combination of bar code reader, touch screen, and keyboard, and initiate the automated processing of the sample. Current optimal design utilizes only reagents which are preloaded in the cartridge.

The instrument will then execute the chemical and biochemical processes of four basic steps (see time chart in previous section):

1. Sample processing
    a. Lysis of cells, opening of viral capsids
    b. Purification of DNA and RNA from proteins and other substituents
    c. Concentration of the sample from a larger volume (250µL) to a smaller volume
    d. Change of the ionic milieu to a low salt solution compatible with RT and PCR steps
2. Reverse transcription (RT)
    a. For RNA targets, a cDNA strand is synthesized using reverse transcriptase, random hexamer primers, and deoxynucleotide triphosphates (dNTPs)
    b. DNA targets will go through this step with little consequence
3. Multiplex polymerase chain reaction (PCR)
    a. Primers for amplification of all of the potential pathogens under interrogation are present in a single reaction (see the list of eight viruses in the Introduction section). *Taq* polymerase and dNTPs are also present
    b. PCR is cycled through 40 cycles of denaturation/annealing/elongation
4. Detection on an electronic microarray
    a. Addressing—electronic hybridization of amplicon mixture to a set of electronically activated pads with unique capture sequences affixed, with different pathogen sequences on those unique capture sites
    b. Reporting—hybridization of fluorescently labeled reporter probes (or bifunctional discriminators as in Figure 1) is accomplished by using a high concentration of these oligonucleotides. This step results in a sandwich forming between the capture probe + target + reporter probe, yielding a fluorescent signal if a target is present
    c. Fluorescent read and data collection—CCD camera read and assignment of certain pixel locations to certain pads, and thus corresponding to the presence or absence of specific pathogens

Instrument Concepts

Figures 5 and 6 show a design of the portable, automated system consisting of a simple to use, touch-screen instrument and a disposable cartridge. The system is miniaturized (ca. 12” x 15” x 15”) and integrates the sample preparation, amplification, and electronic microarray-based detection. All four basic steps described above including sample processing, reverse transcription, multiplexed PCR amplification, and detection on the electronic microarray will be performed within the cartridge. The initial analysis steps comprise: input of a sample, recording of sample identification, and verification of the correct disposable cartridge. The plastic cartridge provides simple fluidics that enables handling milliliter volumes of samples, wash and waste liquids as well as microliter volumes of reagents and eluant. This is achieved using fluidic channels with appropriate diameters depending on sample or reagent volume and a simple linear motion mechanical pumping embedded within the instrument. The system provides a precise manipulation of the reaction piston between the fluidic channels separated by septa as well as dispensing the fluids between the channels and chambers using a plunger. The instrument houses magnets for magnetic bead-based separation and extraction of nucleic acids from the sample. Once the DNA is extracted and eluted into a smaller volume, PCR amplification is performed within a small channel that enables rapid heat and fluid transfer. This concept facilitates very rapid thermal cycling between the heating zones and fast performance of the PCR amplification. The amplified DNA sample is transferred to the detection channel that contains the electronic microarray and detection is performed as in commercial NC 400 system using fluorescently labeled reporters specific for pathogen sequences of interest. The optical imaging is performed using LED optics, lenses, filters and a small CCD camera.

Figure 6 shows a schematic of the instrument concept with a disposable cartridge that is inserted at the top of the instrument. The operation will be fully automated and once the sample is introduced into the cartridge and the cartridge into the instrument, it will require very little hands-on time by the operator. All solutions are contained within the cartridge and no contamination of the operator or instrument is expected.

Fig 5. Conceptual design and operation of the portable automated system – consumable cartridge module.

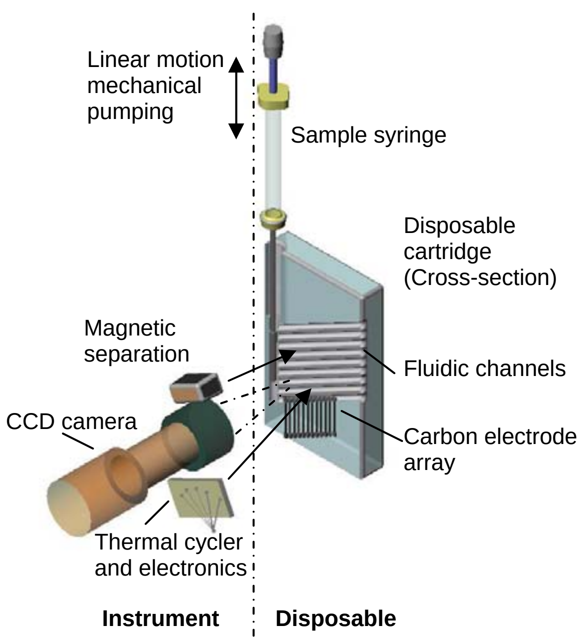
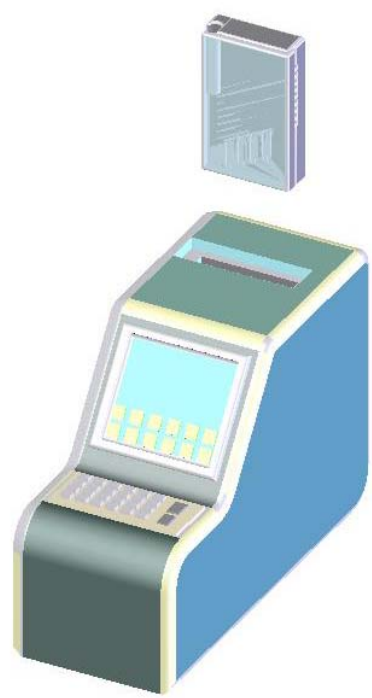


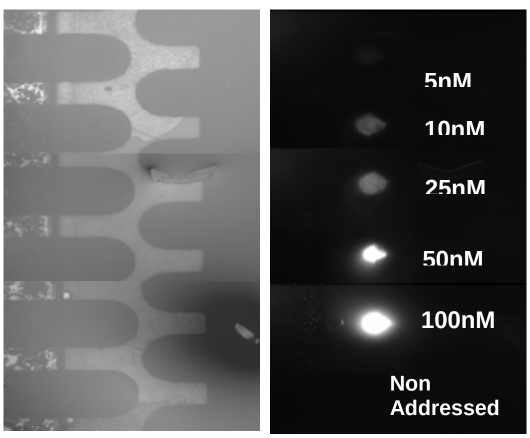
Fig 6. Automated instrument and the disposable cartridge provide full containment of the sample and reagents.



Consumable Cartridge

The cartridge design (cf., Figures 5 and 6) presents a new generation of our low cost disposables, made of plastics and readily manufactured materials. The standard electronic microarray used in the NC 400 system, consisting of platinum electrodes fabricated by silicon micro-machining and photolithography is replaced by plastic substrates and an array of carbon electrodes. The cartridge and the array are fabricated using low cost molding and screen printing processes that will assure >80 fold reduction in manufacturing costs. Figure 7 displays the detection of fluorescence signals on the carbon electrode array. The carbon array and the definition of the electrodes by a dielectric material were fabricated using screen printing on a plastic substrate. A hydrogel permeation layer containing streptavidin in a similar formulation as in the commercial system was deposited on top of the carbon array. Assays were developed and successfully demonstrated on the carbon electrode array for a set of pathogens including *Mycoplasma pneumoniae, Chlamydophila pneumoniae, Streptococcus pneumoniae, Legionella micdadei*, *Bordetella pertussis* and *Staphylococcus aureus.* The assays showed comparable sensitivities to the assays performed on the NC 400 system. Detection of *Streptococcus pneumoniae* and *Legionella micdadei* was successfully demonstrated in a multiplexed assay using a complete set of non-specific captures from our CAP-sepsis panel assay.

Fig 7. White light and fluorescence image on carbon electronic microarray. Addressing of fluorescently labeled SLTII oligo with Cy3 dye (46-mer) – equivalent to amplicon concentrations



White light image **Fluorescence image**

5. Proposed Effort

The project scope can be visually captured by way of the Schedule on page 4. The assumption made is that the starting date is in October 1, 2007. The Specific Aims, above, can be further understood with regard to detail provided here:

1. Definition /risks
   - Market studies, technical risks, financial models
   - IP analysis (currently all IP and licenses appear to be accessible to Nanogen)
   - Specifications development in conjunction with U.S. Department of Defense
2. Proof of Principle
   - Chemistry
   - Test fixture/modules
3. Breadboard Phase
   - Sample preparation, amplification, detection
   - Qualify chemistry and instrumentation
   - Meet all requirements of overall system with a panel of respiratory viruses
4. Integration / Alpha
   - Design control starts in earnest
   - Cartridges – prototype mold, production in thousands
   - Instrument alpha – 10 to 20 units
   - Software – research user version
   - Process development chemistry

– Reagent dispense, reagent drying method development
– Controls development
– Custom robotics to facilitate development throughput (as needed)

5. System Integration / Preproduction
   – Cartridges – higher volume, pre-production tooling
   – Instrument – "packaged"
   – Software – end-customer-like user interface
   – Put system in external lab and run, stand alone, with MTBF ≥ 3 days
   – Full documentation (except the GUI)
   – System customer (market) validation
   – Pilot production line
   – Submit clinical study to CDRH as pre-IDE
   – Incremental panel development begins
   – Distribution plan developed
6. Clinicals
   – Cartridges – higher volume, pre-production/production tooling
   – Instrument – final configuration, pre-production
   – Software – validated, releasable
   – Reagents – 3 unique lots of reagents
   – Validation and verification completed
   – Proficiency testing complete
   – 510(k) / pre-market notification/approval preparation and submission
7. Production Ramp-Up
   – Multicavity hard tools
   – Assembly automation
   – Additional incremental panel development
   – Customer and technical service in place
   – Training
   – Technical sales team assembled
   – FDA clearance process
   – Pre-launch activities

6. Budget Summary

This project is offered to the U.S. Military as a cost sharing program. The estimated cost for both Nanogen and the funding agency is $40 million. Further demarcation of funding can be found on page one of this proposa and following.

Project phases / Statement of Work:

Project Phase	Duration	Key Elements
1. Assay, chemistry feasibility	Start: Month 1 End: Month 12	Bioinformatics, clinical utility for resp. virals, optimization of basic chemistry
2. Specifications, risk analysis	Start: Month 1 End: Month 3	Explicit project specification, contingency planning, IP due diligence
3. Test module development, prototyping	Start: Month 1 End: Month 15	Test fixtures for sample preparation, RT-PCR, and electronic microarrays
4. Breadboard	Start: Month 10 End: Month 21	Linking of test fixtures for 3 primary stages of assay. Demonstration of system utility.
5. Integration / Alpha unit	Start: Month 16 End: Month 27	Design control, prototype molds, production in thousands, 10 to 20 alpha units, software – research user version
6. System integration / Preproduction	Start: Month 25 End: Month 33	Cartridge mfg. volume scale up, design engineering, GUI, external evaluations
7. Clinical trials and validation phase	Start: Month 31 End: Month 39	Clinical trial lots, internal validation and verification, external clinical trial of resp. viral panel, production tooling, 510(k) submitted
8. Production scale up	Start: Month 37 End: Month 42	Multicavity hard tooling, assembly automation, service infrastructure
9. FDA clearance and launch	End: Month 48	(Assumes no special issues with human metapneumovirus due to lack of predicate test)
10. Menu expansion	Start: Month 37 End: Month 48	Feasibility of atypical pneumonia and meningitis panel shown on new platform

The funding estimates pertaining to the phases are:

Project Phase	Nanogen Contribution (including grant funds)	Requested from U.S. Department of Defense	Sum of Cost per phase
1. Assay, chemistry feasibility	$ 1,000,000	$ 850,000	$ 1,850,000
2. Specifications, risk analysis	$ 200,000	$ 0	$ 200,000
3. Test module development, prototyping	$ 2,000,000	$ 300,000	$ 2,300,000
4. Breadboard	$ 2,000,000	$ 1,700,000	$ 3,700,000
5. Integration / Alpha unit	$ 2,000,000	$ 3,900,000	$ 5,900,000
6. System integration / Preproduction	$ 3,250,000	$ 4,900,000	$ 8,150,000
7. Clinical trials and validation phase	$ 3,000,000	$ 4,700,000	$ 7,700,000
8. Production scale up	$ 4,500,000	$ 2,000,000	$ 6,500,000
9. FDA clearance and launch	$ 1,200,000	$ 400,000	$ 1,600,000
10. Menu expansion	$ 1,100,000	$ 1,000,000	$ 2,100,000
SUMS:	$ 20,250,000	$ 19,750,000	$ 40,000,000

The funding requested, on a per year basis (note that several phases cross years) are thus:

Project Year 1 (*i.e.* October 1, 2007 to September 30, 2008)	$1,850,000
Project Year 2	$3,900,000
Project Year 3	$7,000,000
Project Year 4	$7,000,000

This whitepaper proposal is submitted by Nanogen in the expectation that the information herein will be shared and distributed, only as needed to adequately evaluate the proposal and comply with all necessary regulations. All the information is accurate, to the best of our knowledge.

Prepared by:
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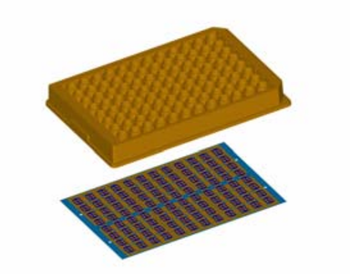
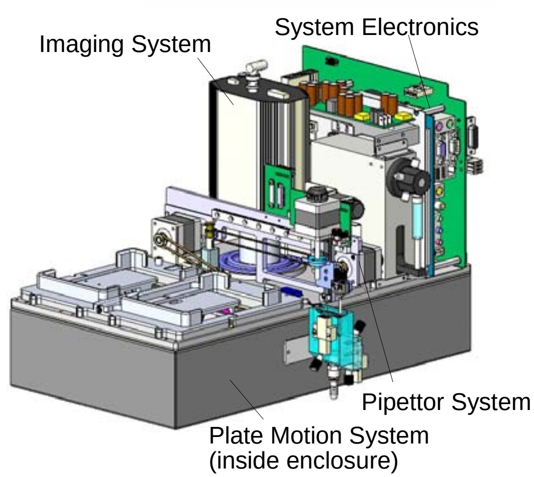
New Developments in Electrochemiluminescence (ECL) Assays for Biowarfare Agents

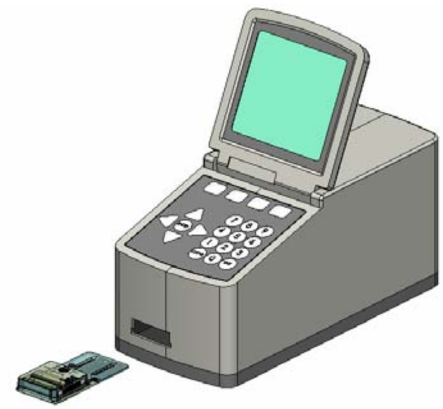
George Sigal
Meso Scale Diagnostics, LLC.
October 26, 2006

Outline of Talk





- MSD Background
  - Multi-Array® Electrochemiluminescence Technology
  - Sector™ Instrumentation
  - Life Science Research Applications
- Biodefense Applications
  - New Instrumentation
  - Detection of Biowarfare Agents
  - Pandemic Influenza



MSD Multi-Array™ Electrochemiluminescence (ECL) Technology

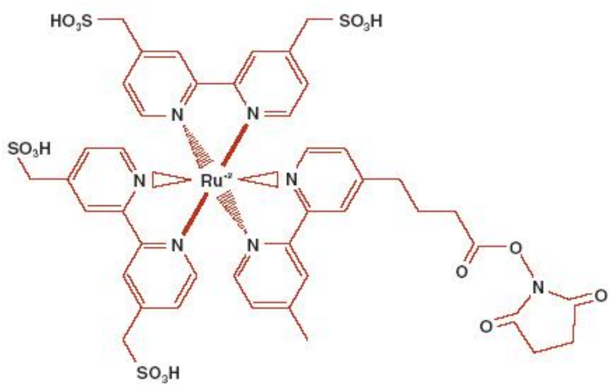
ECL Excitation

Electro- ***chemi-*** ***luminescence***

Electrochemically-driven **Chemical energy** **Emitting light**

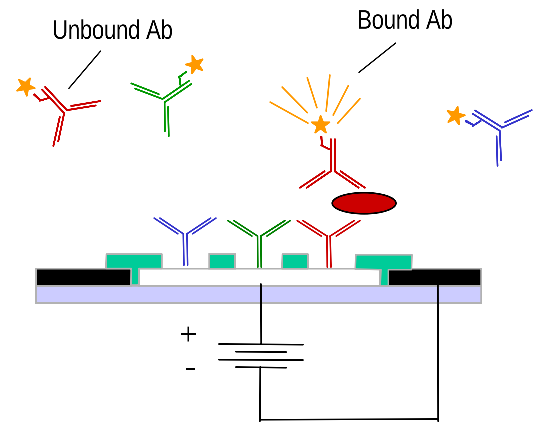
$$\mathbf{N} \xrightarrow{\text{REDOX}} \mathbf{N}^{*} \longrightarrow \mathbf{N} + h\nu$$

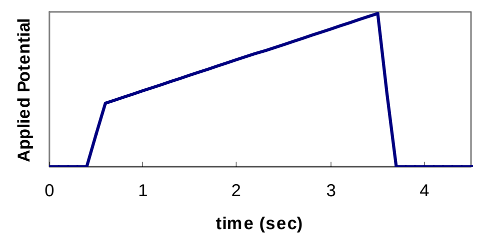
ECL Labels

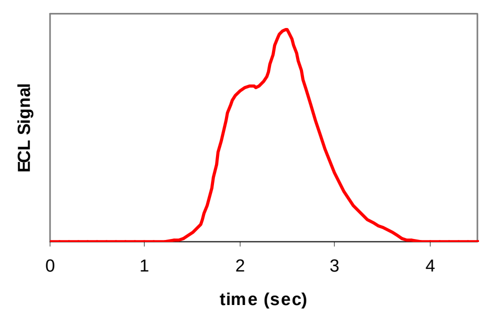


Ruthenium (II) tris-Sulfobipyridine label
(Sulfo-TAG NHS Ester)

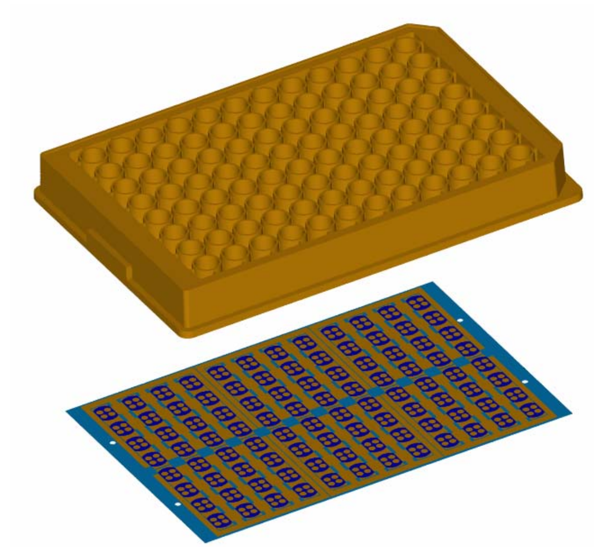
Multi-Array Format

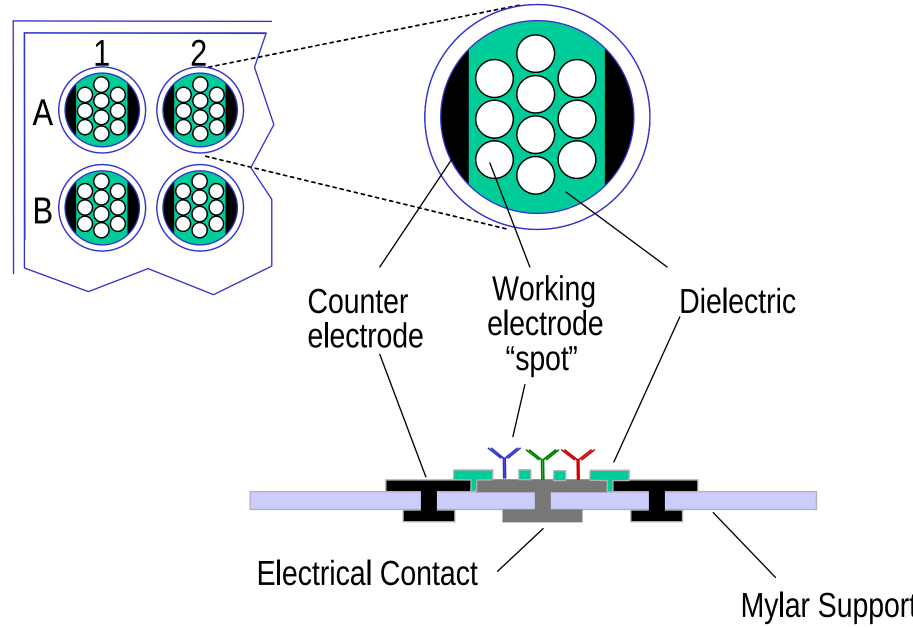




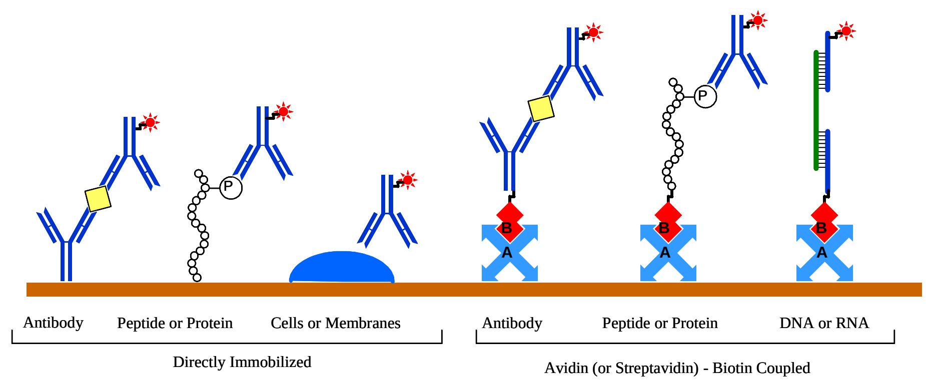


Multi-Array Technology:
Multi-Well Plates With Screen-Printed Electrodes



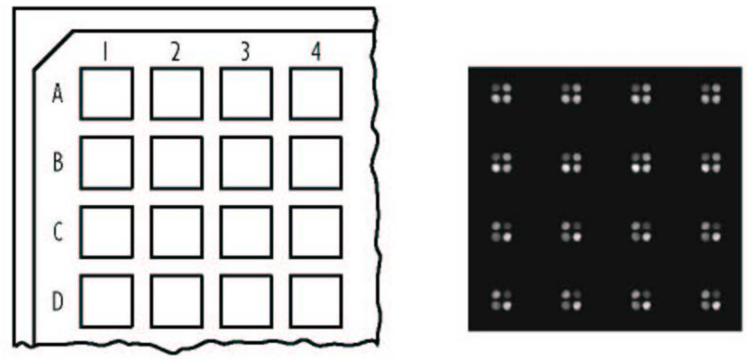


- Single-Plex or a variety of multiplex formats
- Readily scalable manufacturing process
- Carbon ink electrodes can be coated with a variety of biological reagents



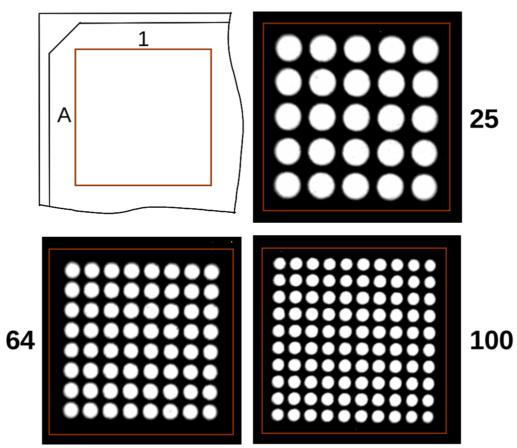
MSD Plates

- Singleplex Measurements in 96, 384 and 1536 well formats
- Multi-Spot Configurations
  - 24-well
  - 96-well
  - 384-well
- Up to 100 “spots” per well



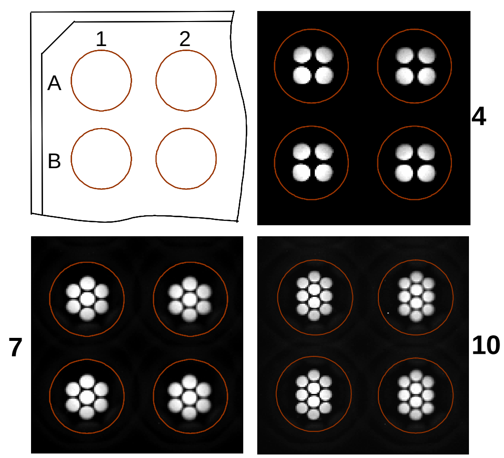
384 wells/plate: 4 spots/well

24 wells/plate, square wells



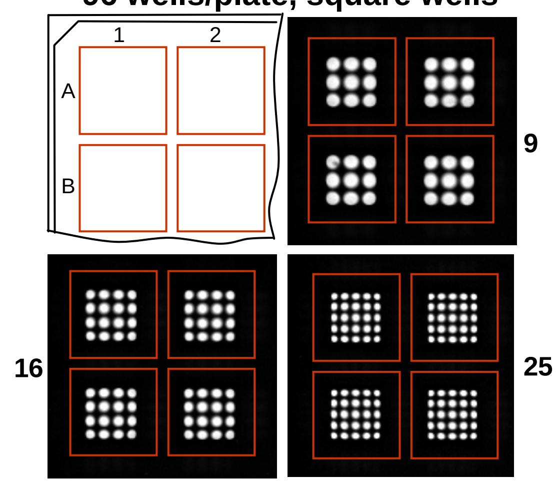
25, 64, and 100 spots/well

96 wells/plate, round wells



4, 7, and 10 spots/well

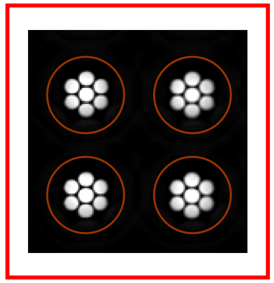
96 wells/plate, square wells



9, 16, and 25 spots/well

MSD Commercial-Off-The-Shelf Instrumentation

Sector™ Imager Instruments



Imaging Detection

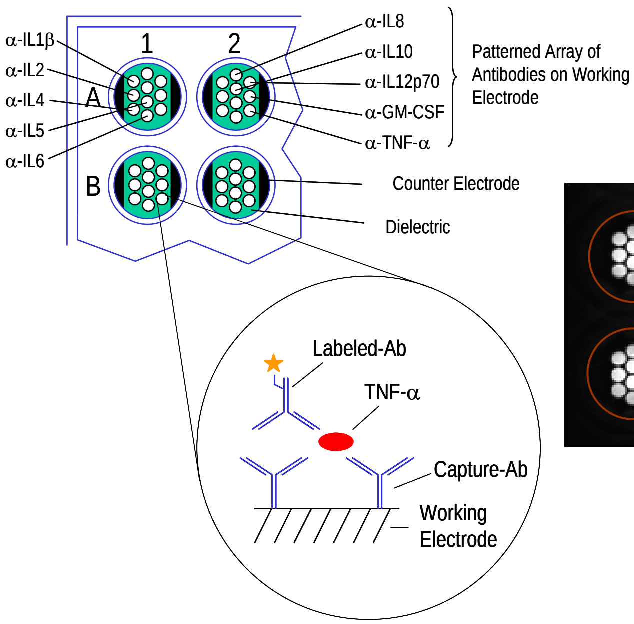
- 24-, 96- and 384-well Multi-Spot plates
- All multiplex formats
  Up to 2500 measurements per plate
- 71 seconds read time per plate
  (independent of level of multiplexing)
- Plate robot compatible
- Integrated plate stacker
- Built-in bar code readers
- Sensitivity: ~ 100,000 protein molecules
- Dynamic range: 10^4 - 10^5

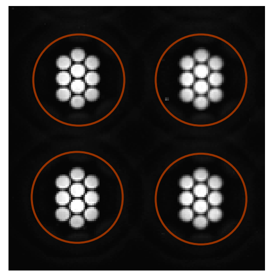
Sector™ PR Instruments

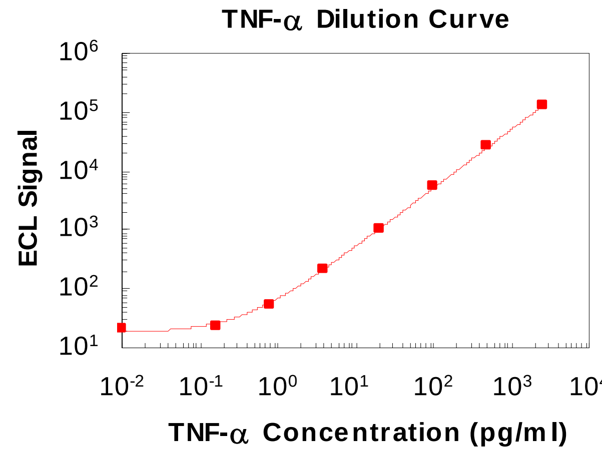
Photodiode Array Detection

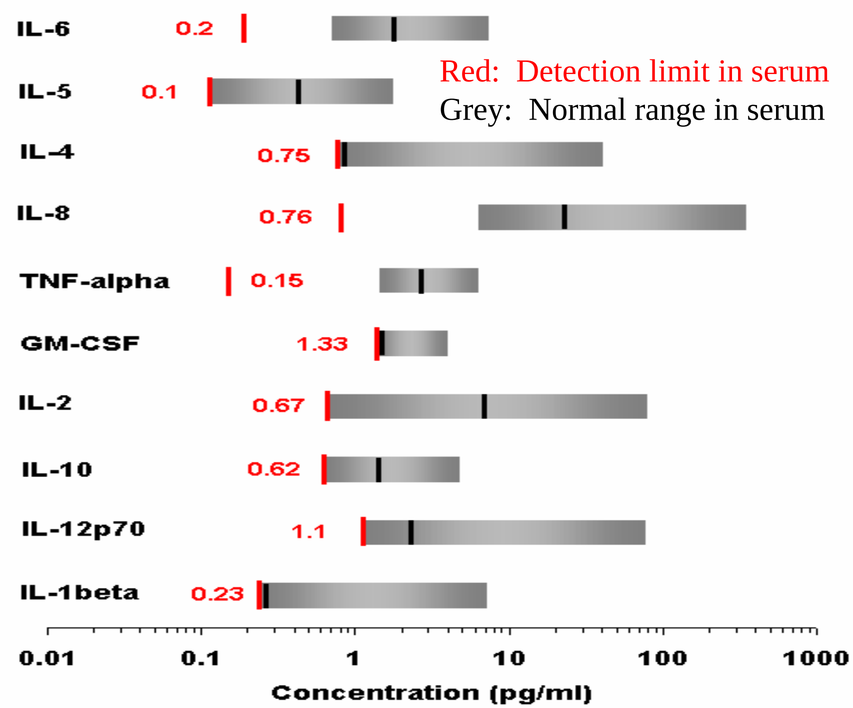
- Portable (~ 18 lbs)
- Can be run from lap-top
- Runs 96-well plates – limited multiplexing
- 2 minutes read time per plate
- Small footprint - 9”(W)x16”(D)x9”(H)
- Touch-sensor operation
- Plate robot compatible
- Built-in bar code readers
- Sensitivity: ~ 250,000 protein molecules
- Dynamic range: 10^4 - 10^5

Sector Imager Performance: Serum Cytokine Measurements









- Excellent performance in clinical matrices
- LODs between 0.1 - 2 pg/mL
- Upper limit of quantification > 10,000 pg/mL
- Spike recoveries in serum at 156 pg/mL
  - Average: 98%
  - Range: 83% - 123%
- 71 seconds per plate

Multi-Array Immunoassay Kits for Life Science Research

Cell Signaling Pathways

Human

Akt	pSer473, pThr308, Total	MEK2	Total
APP	pThr668	Met	pTyr1349, Total
BAD	pSer112, Total	NFκB	pSer536, pSer468
Caspase-3	p20 / p17	p38	pThr180 / pTyr182, Total
EGFR	pTyr1173, Total	p53	pSer15, Total
ErbB2	pTyr1248, Total	p70S6K	pThr421 / pSer 424, pThr389, Total
ERK1/2	pThr202 / pTyr204, pTyr185 / pThr187, Total	PARP	Asp214
GSK-3β	pSer9, Total	PDGFR-β	pTyr751
HIF-1α	Total	S6RP	pSer240 / 244, pSer235 / 236
HSP27	pSer15, pSer78, pSer82, Total	STAT3	pTyr705
HSP70	Total	Tau	pThr231
IGF-1R		VASP	pSer157, pSer 239, Total
JNK	pThr183 / pTyr185, Total	VEGFR2	pTyr1054 / pTyr1059, Total
c-Kit	pTyr721, Total		
MEK1/2	pSer217 / 221, Total		

Assays available in

- Singleplex format
- Multiplexed panels
  - Off-the-shelf
  - Custom user-defined
- In-house kit manufacturing
  - Clean room environment
  - High speed array printers
  - > 120,000 plates / yr

Cytokines and Chemokines

Human

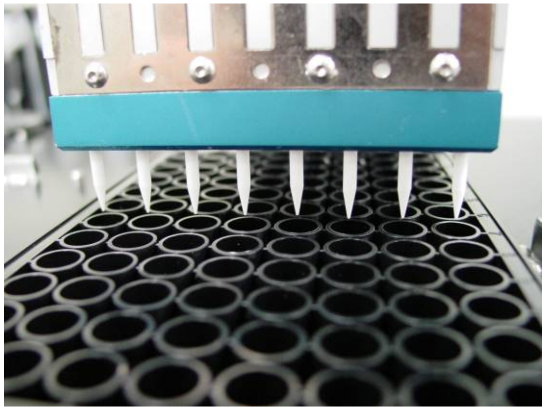
Eotaxin	IL-5	IL-13	MIP-1β
Eotaxin-3	IL-6	IL-17	MIP-3α
GM-CSF	IL-6R	IP-10	RANTES
IFN-β	IL-8	I-TAC	TARC
IFN-γ	IL-10	MCP-1	TNF-α
IL-1β	IL-12	MCP-4	TNF-RI
IL-2	IL-12p40	MDC	TNF-RII
IL-4	IL-12p70	MIP-1α	

Mouse

GM-CSF	IL-12p40
IFN-γ	IL-12p70
IL-1β	KC/GRO/CINC (CXCL1)
IL-2	MCP-1
IL-4	RANTES
IL-5	TNF-α
IL-6	TNF-RI
IL-10	TNF-RII
IL-12	

Rat

GM-CSF	IL-6
IFN-γ	IL-13
IL-1α	KC/GRO/CINC (CXCL1)
IL-1β	MIP-3α
IL-4	TNF-α
IL-5	



Clinical Markers

Alzheimer's Disease

Abeta 40 (Human, Mouse, Rat)
Abeta 42 (Human, Mouse, Rat)
sAPPα (Human)
sAPPβ (Human)
sw sAPPβ (Human)
Phospho-APP (Human)
Tau (Human, Mouse)
β-Secretase (Activity Assay)

Multiplex Panels
sAPPα/sAPPβ (Human)
Abeta Duplex (Human, Mouse, Rat)
Phospho/Total Tau (Human, Mouse)

Vascular Markers & Growth Factors

VEGF (165/121) (Human, Mouse, Rat)
sVEGFR1 (sFlt-1) (Human)
sVEGFR2 (KDR) (Human)
sVCAM-1 (Human)
sICAM-1 (Human)
sICAM-3 (Human)
SAA (Human)
Thrombomodulin (Human)
E-Selectin (Human)
P-Selectin (Human)
HGF (Human)
bFGF (Human)
PIGF (Human)
c-Kit (Human)

Multiplex Panels
Vascular I - sICAM-3, E-Selectin, P-Selectin, Thrombomodulin (Human)
Vascular II - CRP, sICAM-1, sVCAM-1, SAA (Human)
Growth Factor I - bFGF, VEGF, sFlt-1, PIGF (Human)
Growth Factor II - c-Kit, KDR (Human)
Phospho/Total c-Kit (Human)

Cardiac Markers

CRP (Human)
MPO (Human)
Troponin-T (Human)
Troponin-I (Human)
CKMB (Human)
Myoglobin (Human)

Multiplex Panels
Cardiac - CKMB, Myoglobin, Troponin I (Human)

Fertility Markers

FSH (Human)
LH (Human)
Progesterone (Human)

Multiplex Panels
Fertility - LH, FSH, Progesterone (Human)

Metabolic Markers

Adiponectin (Mouse)
GLP-1 (Human, Mouse, Rat)
Insulin (Human, Mouse, Rat)
Leptin (Mouse)
Resistin (Mouse, Rat)

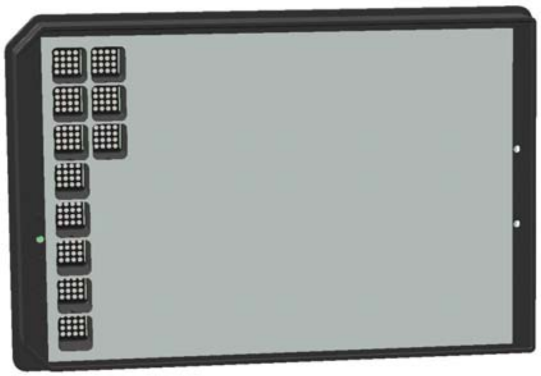
Multiplex Panels
Metabolic - Leptin, Insulin (Mouse)

Hypoxia Markers

VEGF (165/121) (Human, Mouse, Rat)
EPO (Human, Mouse, Rat)
HIF-1α (Human)
IGFBP-1 (Human)

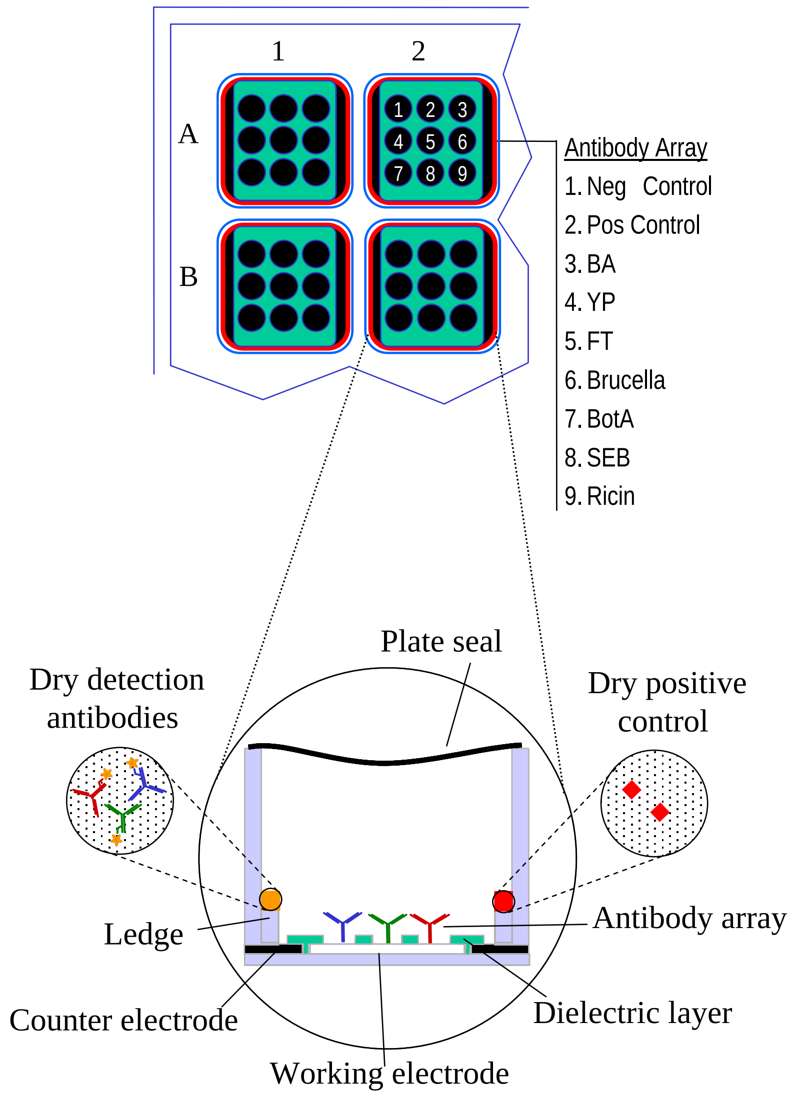
Multiplex Panels
Hypoxia - EPO, VEGF (Mouse, Rat)
Hypoxia - EPO, IGFBP-1, VEGF (Human)

Biodefense Applications: Assay Formats

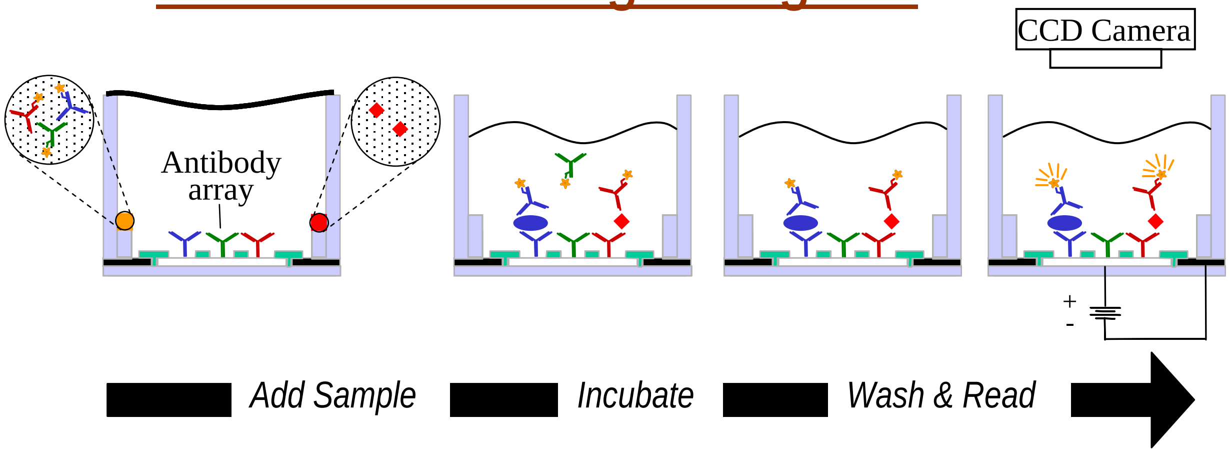


New Square Well Plate Format

- 9, 16 or 25 spots per well
- All biological reagents stored dry in well
- Built in ledge for dry detection reagents
- Foil seal and optional integrated desiccant for long term stability out of package
- Integrated positive and negative performance controls
    - Negative: Unpaired capture antibody
    - Positive: Full sandwich assay for spiked control analyte



Biodefense Applications: Multiplexed Detection of Biological Agents

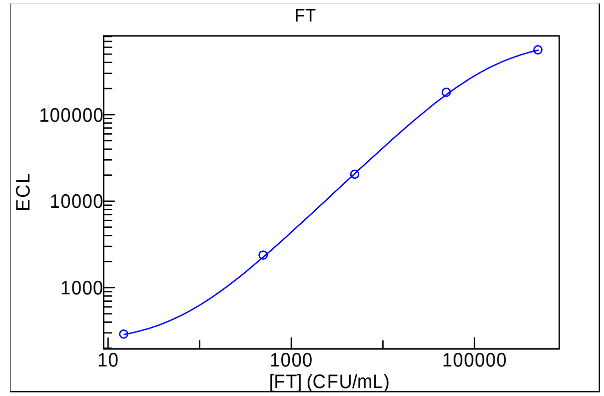


Target	Limit of Detection
Bacillus anthracis (BA)	14,000 CFU/mL
Brucella spp.	11,000 CFU/mL
Francisella Tularensis (FT)	20 CFU/mL
Coxiella Burnetii (Q Fever)	1,300,000 CFU/mL
Yersinia Pestis (YP)	180 CFU/mL
Vaccinia	17,000 PFU/mL
Venezuelan Equine Encephalitis (VEE)	93,000 PFU/mL
Botulinum A Toxin	5 pg/mL
Ricin	2 pg/mL
Staph Enterotoxin B (SEB)	0.4 pg/mL

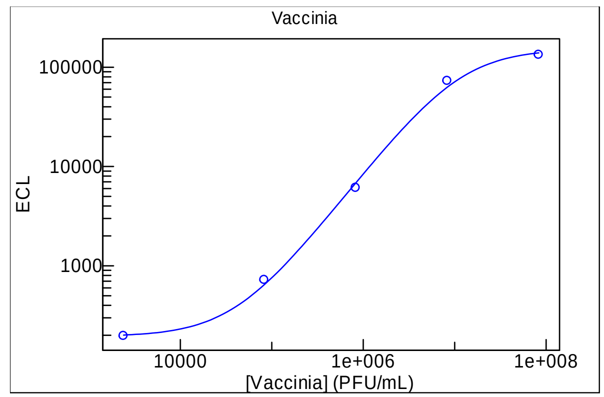
Limit of Detection is concentration that gives a signal 2.5 SD above background

Antibodies primarily from Critical Reagents Program and Tetracore
Run on Sector Imager 6000 as 2 x 5-plex panels, samples in clean buffer, 1 hr incubation
Detection limits may vary for different antigen preparations

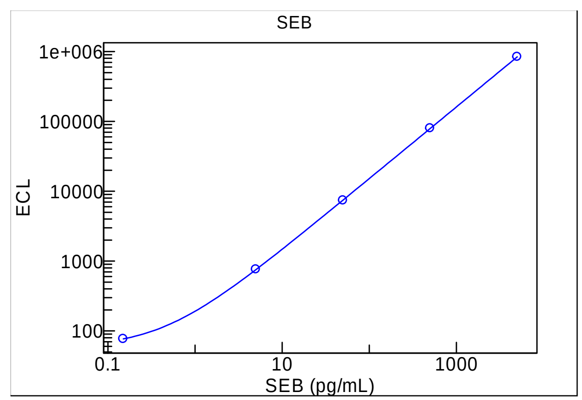
Bacteria



Viruses



Toxins

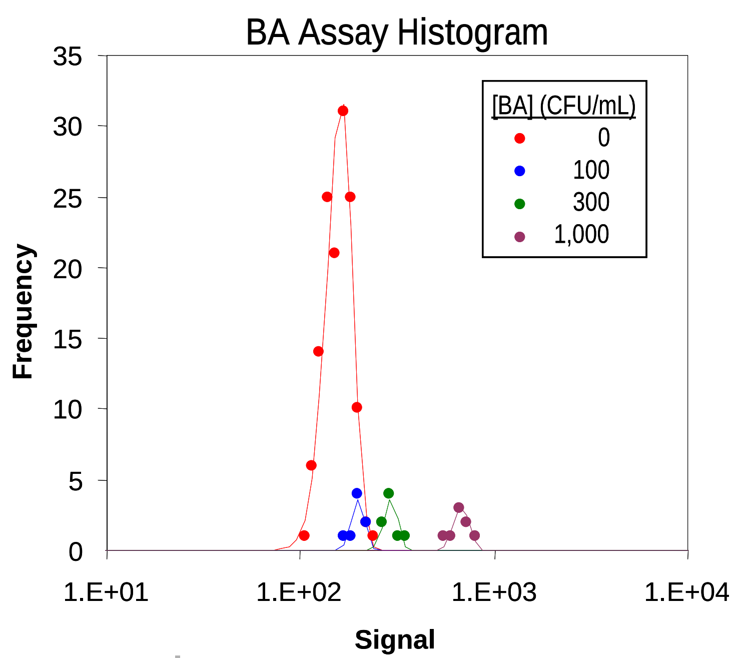


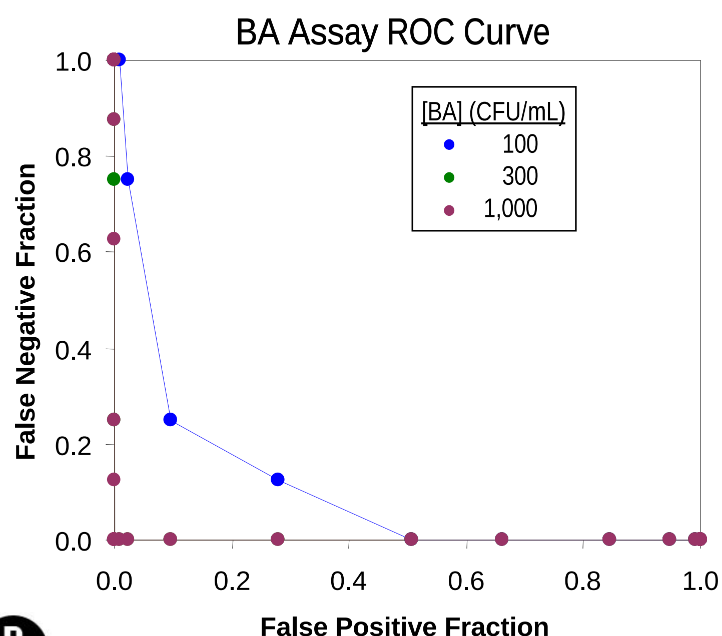
BWA Detection: ROC Analysis

- Testing carried out by ECBC Biosensors Group
- Tested 320 samples, each in triplicate
  - 136 zeros and 24 positives for each analyte
  - Three different positive levels for each analyte
  - Positives for each assay used as zeros for others
- Results based on data from 10 plates measured over multiple days
- Histograms show distribution of signals for each sample type; data fit to Gaussian distribution
- ROC curves plot FPR and FNR for different thresholds

BA Assay Statistics

	[Agent] Zero	Low	Mid	High
Concentration				
CFU/mL	0	100	300	1,000
Assay Signals				
Signal Ave	151	190	279	646
Signal Stdev	24	15	21	56
# of Samples	136	8	8	8
ROC Analysis				
Threshold	-	182	240	240
FPF	-	0.096	0.000	0.000
FNF	-	0.250	0.000	0.000
ROC Area	-	0.106	0.000	0.000





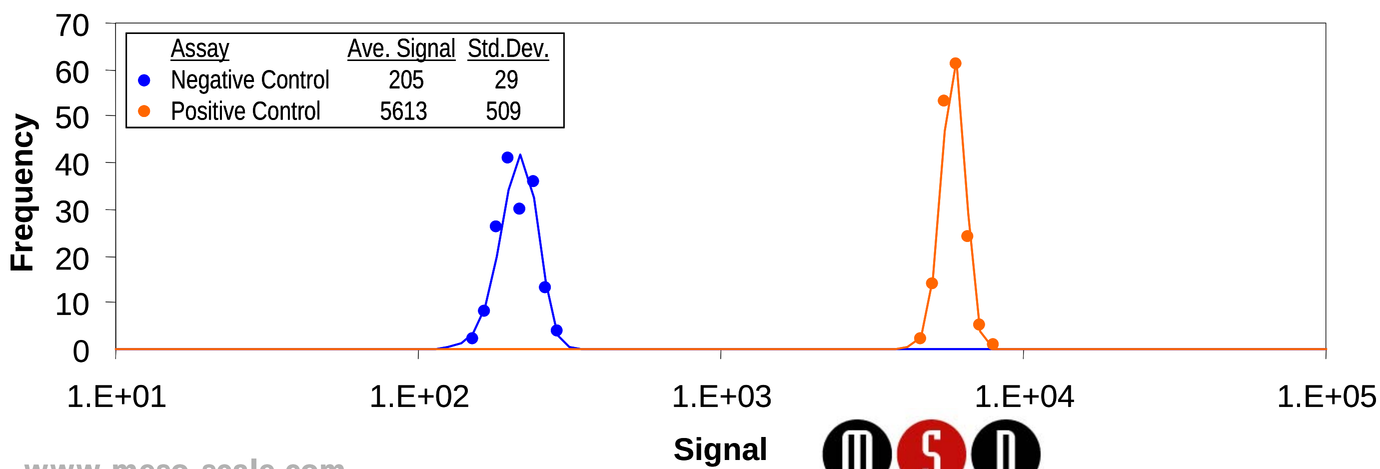
BWA Detection: ROC Analysis

Test Assay Results

Assay		Zero Signal	Zero Std. Dev.	Spiked [BWA]	Spiked Units	Spiked Signal	Spiked Std.Dev.	ROC Results Threshold	FPR	FNR	Area
***Bacillus anthracis* (BA)**		151	24	**300**	**CFU/mL**	279	21	240	0.0%	0.0%	0.000
***Brucella* spp.**		346	41	**3,900**	**CFU/mL**	1012	35	501	0.0%	0.0%	0.000
***Coxiella burnetii* (Q Fever)**		337	39	**120,000**	**Part./mL**	520	38	457	0.0%	0.0%	0.000
***Francisella tularensis* (FT)**		1003	173	**690**	**CFU/mL**	2012	188	1500	0.0%	0.0%	0.000
***Yersinia pestis* (YP)**		191	36	**270**	**CFU/mL**	795	64	288	0.0%	0.0%	0.000
Vaccinia		115	18	**26,700**	**PFU/mL**	238	14	182	0.0%	0.0%	0.000
Venezuelen equine encephalitis		248	47	**4,800,000**	**PFU/mL**	446	26	380	0.0%	0.0%	0.000
Botulinum A Toxin (BotA)		53	15	**17**	**pg/mL**	122	5	95	0.0%	0.0%	0.000
Ricin		121	29	**3**	**pg/mL**	218	21	182	2.2%	0.0%	0.003
Staph Enterotoxin B (SEB)		179	31	**2.7**	**pg/mL**	320	30	240	1.5%	0.0%	0.001

[BWA] is the lowest concentration tested that was detected with at least 95% accuracy
Threshold is the optimal threshold be ROC analysis
FPR and FNR are the false positive and false negative rates
Results may depend on source of antigen

Control Assay Results



BWA Detection: Interference Analysis

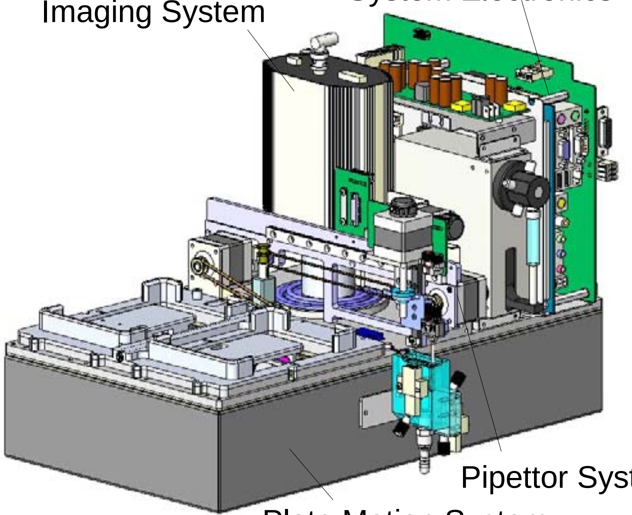
- Testing carried out by ECBC Biosensors Group
- Tested against interferents supplied by DoD Critical Reagents Program
- Tested blank samples and samples spiked with BWA antigen preparations
- Pass criteria is < 50% increase in background or decrease in specific signal for undiluted interferent solution

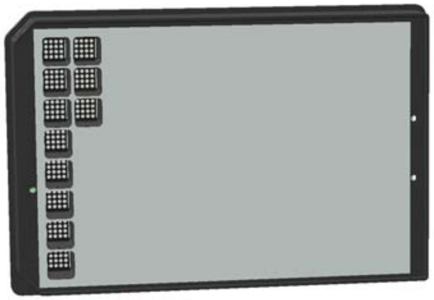
	Assays									
Interferent	**BA**	**BotA**	**Brucella**	**FT**	**Q Fever**	**Ricin**	**SEB**	**Vaccinia**	**VEE**	**YP**
A. niger	✓	✓	✓	✓	✓	✓	✓	✓	**X**	✓
BSA	✓	✓	✓	✓	✓	✓	✓	✓	**X**	✓
Burn Veg.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Burn. Diesl.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clay Soil	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Malathion	✓	✓	✓	✓	✓	✓	✓	✓	**X**	✓
Water	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

- Only significant interference was observed reduction in VEE signals in several interferents
- May be related to VEE preparation; further investigation in progress

Biodefense: New Instrumentation – PR2 Multiplexed Plate Reader and Processor

- Compact plate reader (12”x17”x13”, < 35 lbs)
- Imaging system provides multiplexing capability and sensitivity of Sector Imager in much smaller size
- Integrated sample station and pipetting system for fully automated sample analysis
- Integrated seal-piercing tool for unsealing and using one assay well at a time
- Up to 1-month walk-away operation – integrated plate stacks, low reagent use, stable consumables
- Applications:
    - Detector for autonomous air samplers/analyzers
    - Automated laboratory analysis
    - Food testing
- Used in Technology Readiness Evaluation (Water Testing) at Dugway in June 2006
- External evaluation underway at NMRC

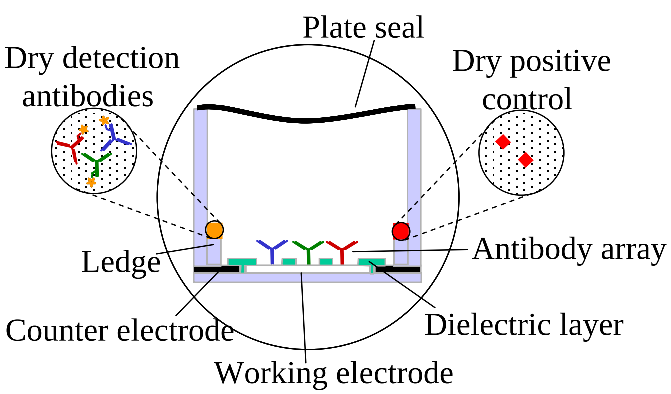


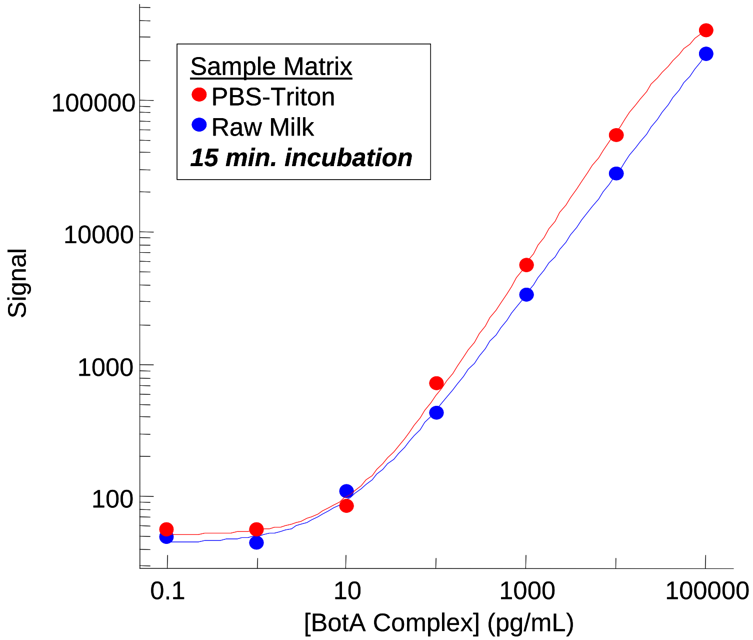


PR2 Results: Measuring Bot A Toxin in Raw Milk

Assay format

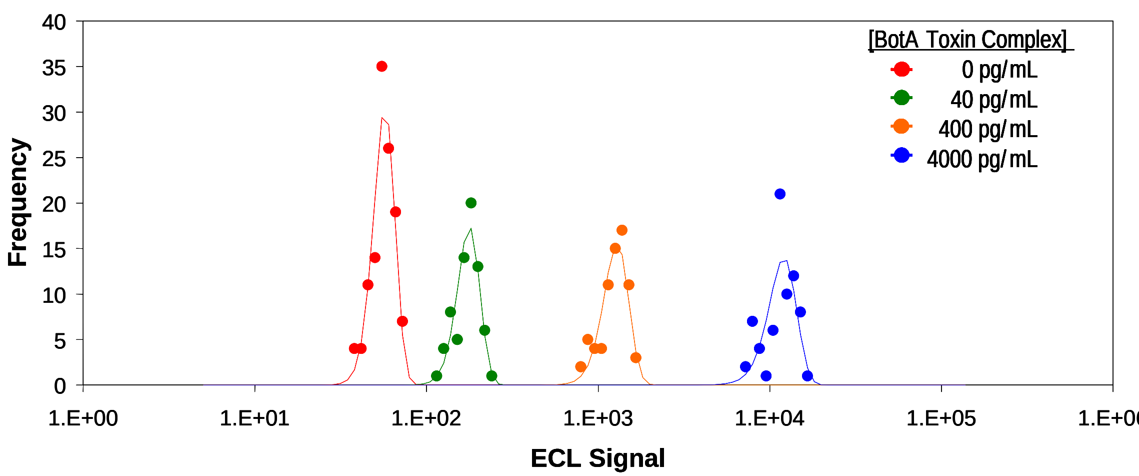
- Dry reagent format
- No sample preparation required
- 125 uL sample, 15 min. incubation
- No matrix effect from raw mil
- Detection limit of about 17 pg/mL





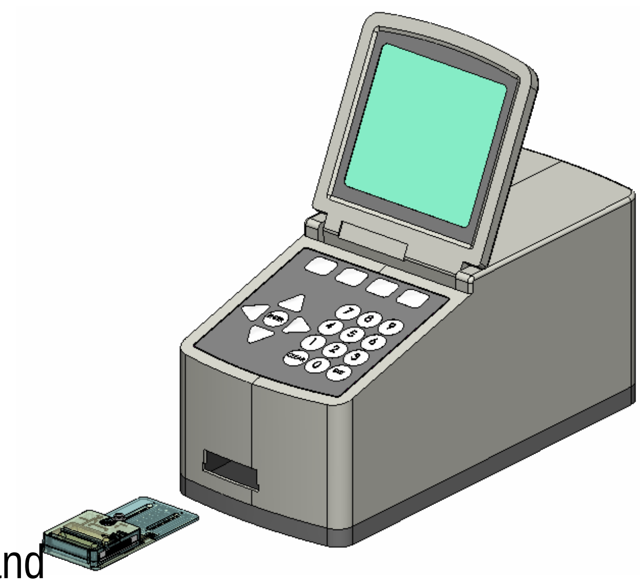
ROC Analysis

- Spiked raw milk samples
- Complete discrimination of 0 and 40 pg/mL BotA toxin

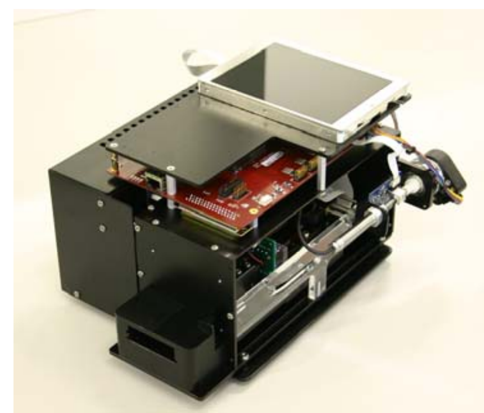


New Instrumentation: ECL Cartridge-Based POC Diagnostics

- Prototype refinement under NIH funding for direct and serological measurements of biothreat agents.
- Disposable single use injection molded cartridge with integrated fluidics for fully automated processing of one sample.
- Portable cartridge reader for analyzing cartridges
- Fully automated processing
- Multiplexed ECL detection using the same screen printed electrode technology in MSD’s plate based systems.
- Two cartridge designs for liquid (such as blood and urine) and solid (swab) samples.
- Fluid movement in cartridge controlled using air pressure/vacuum and optical sensors
- Less than 15 min to result
- Reader size: 7”x7”x12” (HxWxD)
- Reader weight: < 10 lbs
- Power consumption: < 30 Watts; battery operation available
- Cartridge and reader system will be advanced under CDC contract for pandemic flu diagnostics

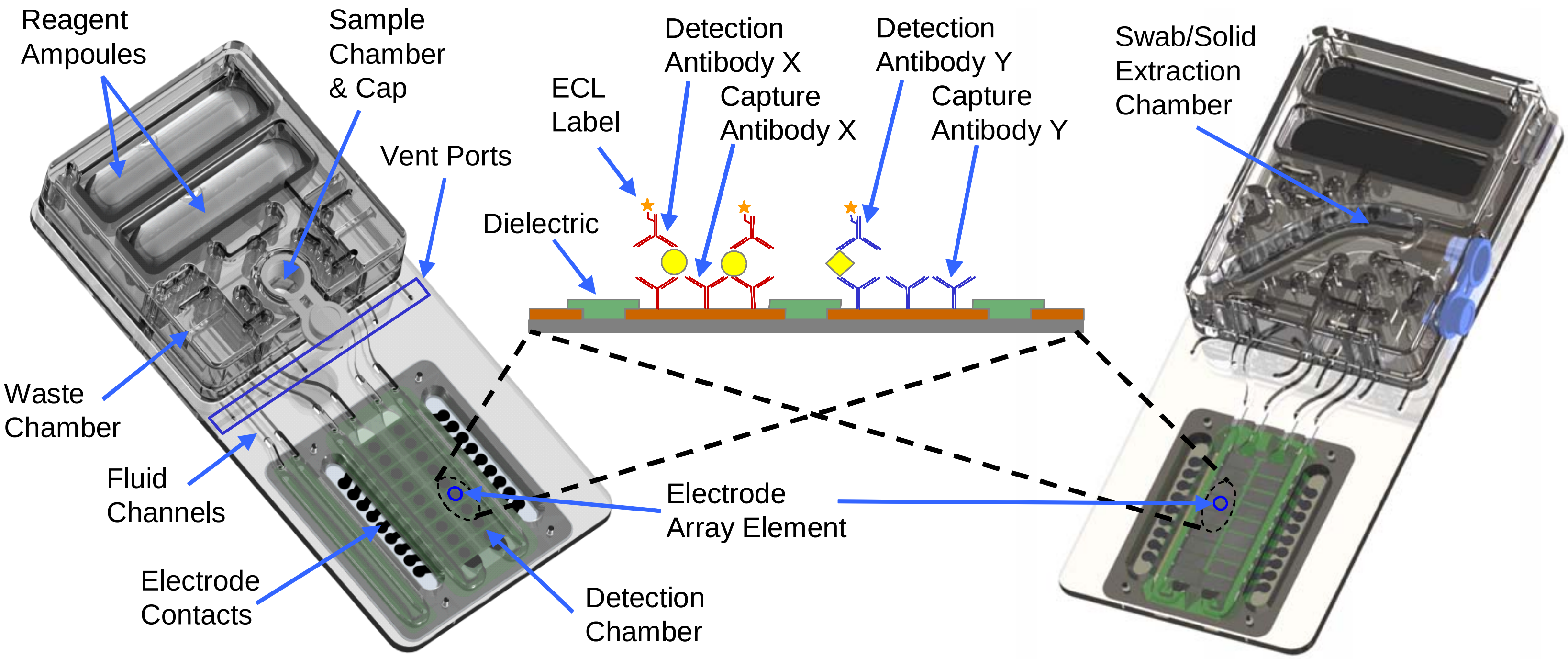


Cartridge Reader Model



Cartridge Reader Prototype

Cartridges with Integrated Microfluidics

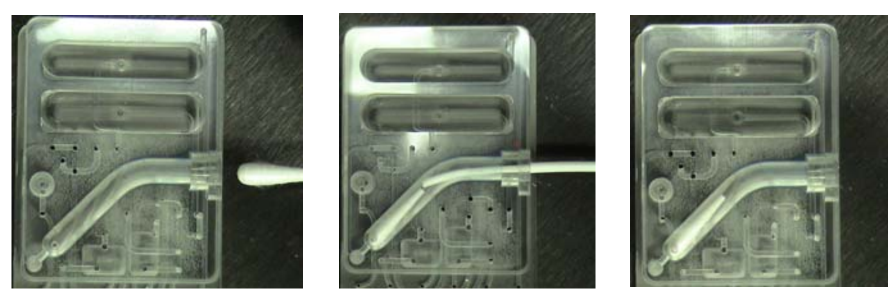


Liquid Sample Cartridge **Solid/Swab Cartridge**

Features

- 8–10 capture zones/channel
- 2 independent channels
- Dry biological reagents
- Liquid wash/read buffer
- Two-step format (optional)
- Versatile micro-fluidic design

Swab sample chamber is designed to break off swab head

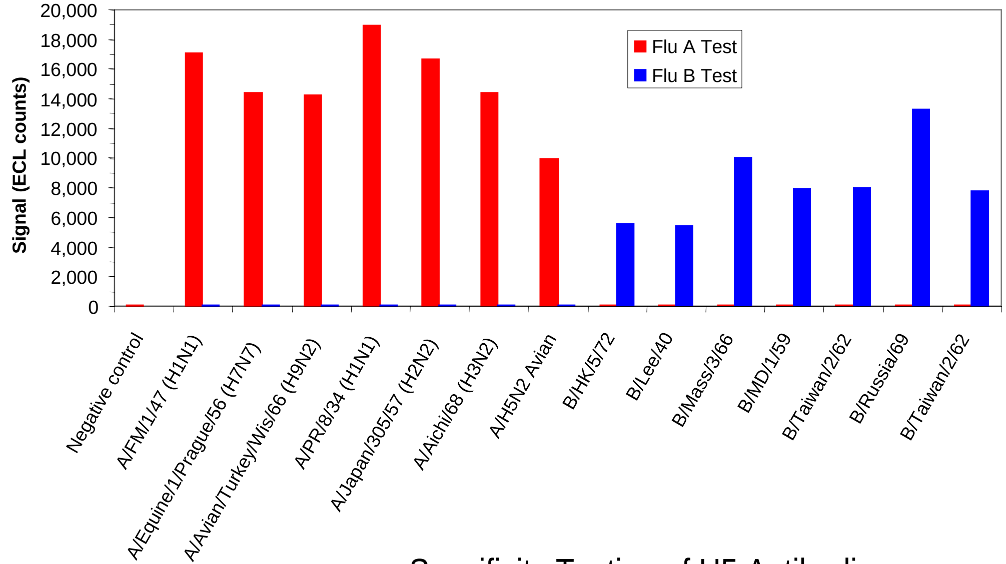


Pandemic Influenza Detection: Antibody Selection

Assays for Flu A and B Nucleoproteins

- Selected antibodies for
  - Type specific detection of influenza
  - Broad recognition across strains/subtypes
- Specificity testing against viral isolates carried out in plate format

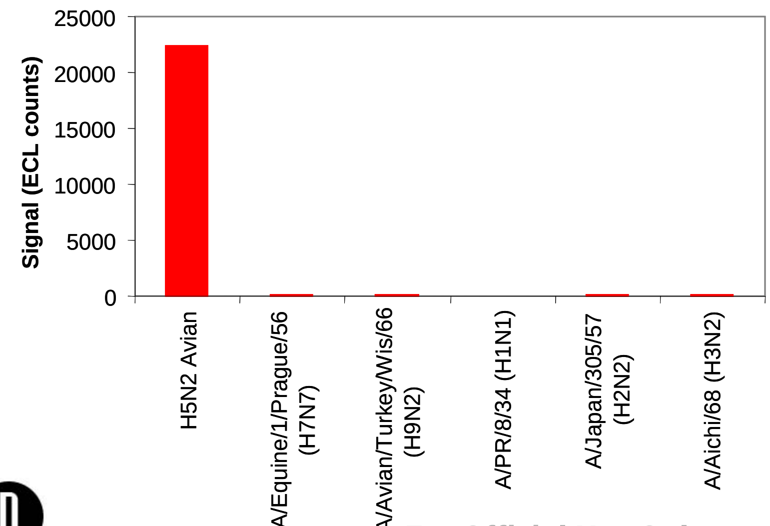
Specificity Testing of FluA and FluB Nucleoprotein Antibodies



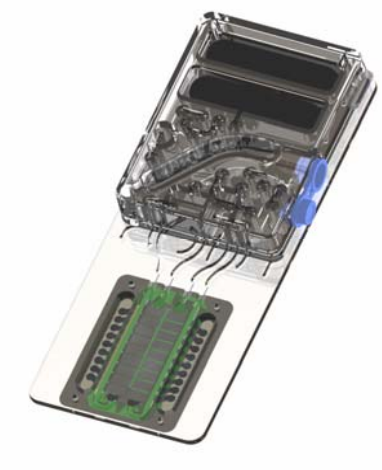
Assays for H5 Influenza Hemagglutinin

- Selected antibodies for
  - Subtype specific detection of H5 hemagglutinin
  - Broad recognition of different H5 strains
- Specificity testing against recombinant hemagglutinins carried out in plate format

Specificity Testing of H5 Antibodies



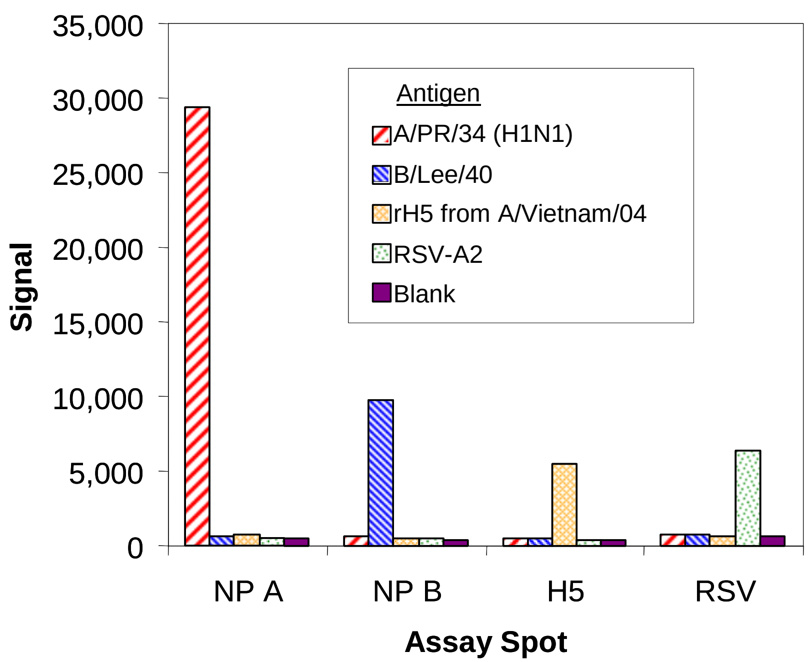
Pandemic Influenza Detection: Swab Panel Sensitivity and Specificity



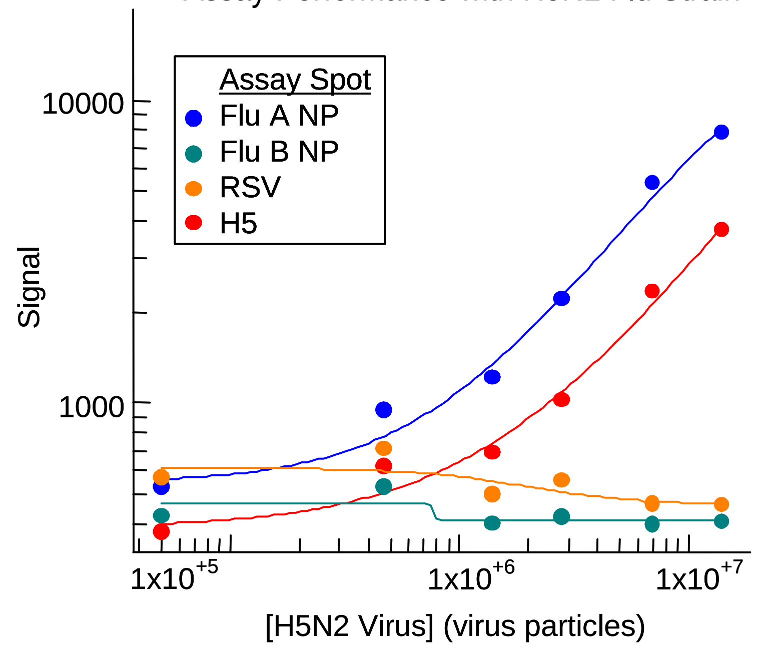
Swab Cartridge Upper Respiratory Infection Panel

- Panel includes assays for Flu A and B nucleoprotein, Flu H5 subtype and RSV
- Testing used antigen spiked onto swabs
- Automated swab extraction and analysis carried out with swab cartridge and cartridge reader
- Specificity demonstrated with Flu A (H1N1), Flu B, RSV isolates and recombinant H5 from high pathogenicity H5N1 strain (left) and low pathogenicity Flu A (H5N2)

Specificity Testing of Upper Respiratory Panel



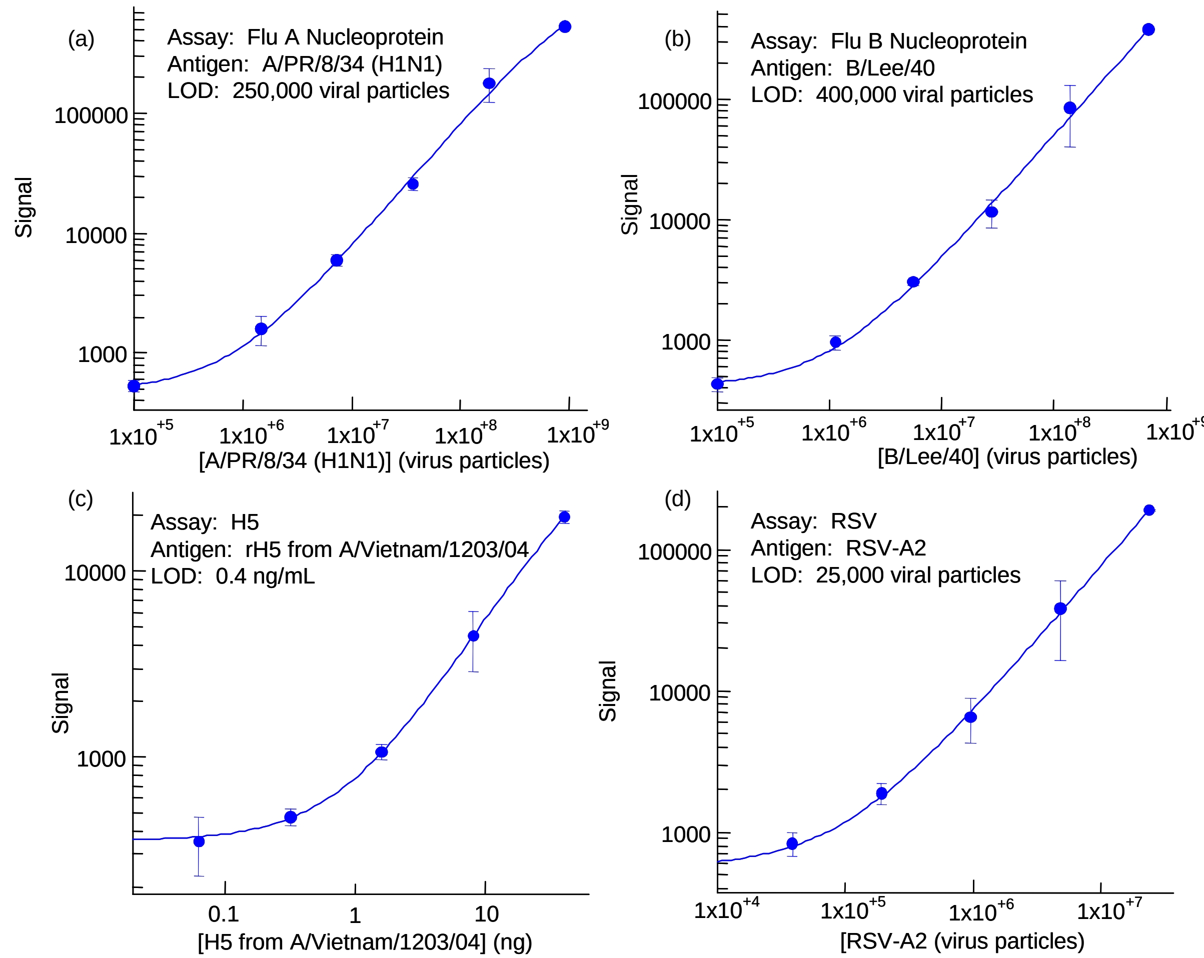
Assay Performance with H5N2 Flu Strain



Pandemic Influenza Detection: Swab Cartridge Assay

Swab Cartridge Upper Respiratory Infection Panel

- Flu A and B nucleoprotein, Flu H5 subtype and RSV
- Antigen spiked onto swabs
- Automated swab extraction and analysis carried out with swab cartridge and cartridge reader
- Plots show results for FluA, FluB and RSV viral isolates and recombinant H5 protein
- Assays are 10-100 times more sensitive than most sensitive strip tests
- Strip tests used according to manufacturers instructions
- Range of LODs for strip test covers range of “possibly positive” to “clearly positive” strip results



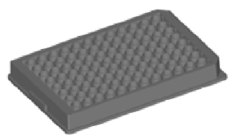
	LOD (Viral Particles)	
	Flu A	**Flu B**
MSD Cartridge	2.5x10^5	4x10^5
Commercial Strip Test	7x10^6-3.5x10^7	6x10^6-2.8x10^7

The MSD Family of Instrumentation

- One Detection Technology – Many Flavors of Instrumentation
- High Throughput Lab Testing to Field Analysis
- Simplified Assay Development – Uniform Performance Across Testing Environments

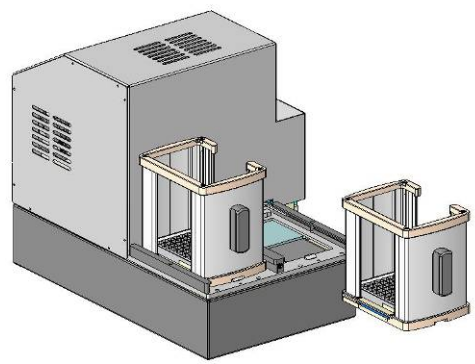
High-Throughput Lab ↔ Field Lab ↔ Field

Sector Imager (COTS)

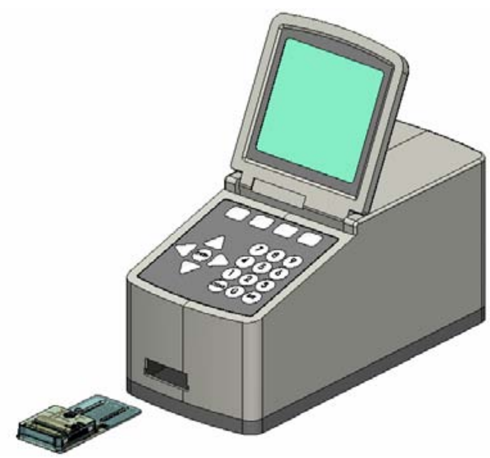


Multi-Array Plate

Sector PR (COTS)



PR2 Instrument



Multi-Array Cartridge and Reader

Plate Based

Cartridge Based

Acknowledgements

MSD Team

Government Collaborators

- Edgewood Chemical and Biological Center (ECBC), Biosensors Group
  Deborah Menking, Kishna Mangaya, Bruce Voelker
- US Army Medical Research Institute for Infectious Diseases (USAMRIID)
  Cindy Rossi, Jeanne Geyer
- Navy Medical Research Center (NMRC)
  Jill Czarnecki

Reagents Providers

- DoD Critical Reagents Program
- Tetracore, Inc.
- Biodefense and Emerging Infections Research Repository

Funding Agencies

- DoD
- HSARPA
- NIH/NIAID

Meso Scale Diagnostics, LLC.

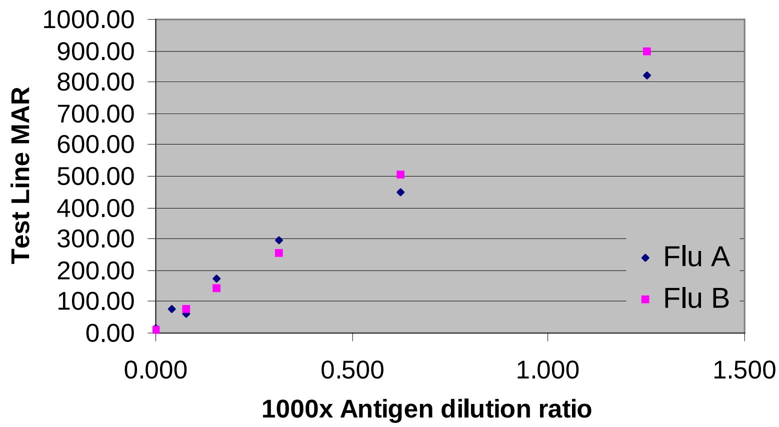
Influenza Assay Device Development Summary

MagnaBiosciences is in the development of MICT Flu A/B assay devices for influenza A and influenza B antigen detection in nasal and throat swab samples. Current device prototypes can detect low level of influenza viral culture that can not be detected by other commercialized rapid test kits.

		Flu A		Flu B	
dilution	1000x dilution ratio	Test	Control	Test	Control
1 to 800	1.250	820.40	1495.50	896.3	696.15
1 to 1600	0.625	449.00	1196.00	503.7	616.95
1 to 3200	0.313	296.40	1897.60	256.75	633.45
1 to 6400	0.156	173.90	2073.10	144.2	716.1
1 to 12800	0.078	62.30	1589.10	76.4	668.7
1 to 25600	0.039	75.10	2542.90		
Buffer	0.000	12.80	1849.60	10.95	741.35

Competitor Rapid Assay Kit Detection Limit

Detection of Influenza A and B Antigens By MICT FluA/B Devices



Portable Biological Assistance Capability for Protecting A Population (PBA)
White Paper to Accompany Preproposal Document

for HQ AF/SGR BAA 06-1

Section A:

Title: Portable Biological Assistance Capability for Protecting A Population (PBA)

Period of Performance: Phase I (Feasibility) 12 Months, Phase II (Tested, Manufacturable Prototype) TBD based upon refinements developed during the feasibility study (Estimated time based upon current requirements 24 Months).

Estimated Cost of Task: Phase I $2,000,000.00, Phase II TBD based upon refinements developed during feasibility study (Estimated Cost based upon current requirements: $8,000,000.00)

Name of Institution: The Center for Applied NanoBioscience, at the Biodesign Instititue, Arizona State University.

Section B:

Task Objective: The objective of the proposed research and development, over the long term, is to demonstrate a portable platform for automated multiplexed profiling of 100 pathogens in less than 2 hours. The objective of the first phase of this project is to develop a limited prototype that demonstrates the feasibility of integrating the core technology for pathogen profiling into a stand alone portable platform. The second phase of the project will develop a ready-for manufacture prototype.

Section C:

Technical Summary/Abstract:

The success of the United States Armed Forces in theater engagements, humanitarian aid, and disaster management is contingent upon the maintenance of personnel in a robust state of operational readiness. Any threat reducing the number or readiness of effective personnel must be dealt with quickly and efficiently. The greatest historical challenge has been presented by biological disease. As a result, military forces have longstanding critical requirements for tools that can identify whether individuals are infectious (so that decision makers can treat and, if necessary, isolate them). Military forces also require monitoring systems (consisting of collection, analysis and information dissemination capabilities) that can help decision makers understand the nature and potential effects of the threat. In order to address this clear critical and present need, NMRC and the Center for Applied NanoBioscience at the Biodesign Institute will combine their capabilities in live agent content development, assay chemistries, packaging and integrated micro-systems processing to create an automated portable biological detection system (PBA)

based on multiplex molecular assays. The PBA comprises a micro-fluidic programmable array platform for measuring signatures of gene expression biomarkers from blood samples as well as protein and toxins from saliva. The PBA has the potential to allow early and definitive diagnosis of exposure to infectious agents (particularly agents that may be used by bioterrorists), can be used at the site of any presumed exposure, can be operated by minimally trained personnel with a turn around time of potentially less than 2 hours, enables the high sample throughput necessary for scalable screening and validation of forces at risk, and strives for greater robustness and simplicity at a lower cost than PCR assays.

Background:
There is an operational need for earlier, more definitive identification of personnel exposed to infectious agents. Ideally the diagnosis of exposure needs to be made before or at the earliest onset of symptoms. The measurements of molecular biomarkers from saliva and the cellular elements of the blood provide the opportunity for presymptomatic detection and diagnosis. The enabling platform must allow testing to be performed by minimally trained personnel at the point of presumed exposure rather than in a remote reference lab by highly trained technicians. The EOS program is using high density arrays to identify gene expression biomarkers of infection. The diagnostic platform on which to run these biomarkers needs to be developed. The PBA platform will be developed using the following specific attributes:

1) Gene expression biomarkers for selected high priority diseases
2) Multiplexed assays to signatures of the infectious agent
3) "Sample-to-answer" turn around time of less than 4 hours (potentially less than 2 hours)
4) Greater robustness and simplicity, with similar sensitivity, at a lower cost than PCR
5) Ability to detect smaller, and thus potentially earlier, changes in gene expression levels than PCR
6) Push-button platform (add sample, push button, get definitive result without need of interpretation by a physician)

Objective/Hypothesis: There are no available platforms for the multiplexed, sensitive measurement of gene expression signatures from saliva samples or blood cells with the simple sample collection and stabilizing options available using qNPA. Nor do there exist platforms with the well-integrated, simple automation of sample preparation and protocol, the accuracy and sensitivity to measure small changes in gene expression or the necessary "Sample-to-answer" turn around times with the robustness requisite of field-based measurement. Our proposed integrated microfluidic platform (PBA) will allow detection of 100 pathogens/ run using biomarkers of 5 genes/pathogen on an array of 500 elements. The assay platform will provide direct pathogen detection (rRNA/DNA) as well as host cell biomarker detection (RNA/protein). The cycle time from sample input to diagnostic result will be less than 6 hours (current benchtop configuration) and potentially less than 2 hours (microfluidic configurations with smaller volume and mixing). The platform will be expandable to measure new biomarker signatures while it will also be amenable to the accommodation of a protein-based assay for rapid triage

(<15 minutes). The system will be integrated as a portable reader for field use by an Independent Duty Corpsman. A unique feature of the proposed system is the capability to use formalin fixed (stabilized) samples without refrigeration or freezing which also provide logistics value for field operation. It is anticipated the proposed platform can satisfy most of the JB3 and EOS requirements.

Study Design: To reduce risk to the Navy, the overall project is divided into (1) a Feasibility Phase (estimated cost: $2,000,000.00) and (2) a Development Phase (estimated cost: $8,000,000.00). The first (feasibility) phase is directed towards concept validation and production of an alpha-test PBA. The demonstration of the -test PBA platform will utilize an array of 25 elements and the measurement of white cell genes from 200 ul of blood with a turn around time of less than 6 hours. In the second (development) phase, the array size will be increased (e.g. 500 elements) to detect exposure to the desired number of pathogens (e.g. 100), the sensitivity will be increased, the turn around time decreased (potentially to less than 2 hours), reagent storage will be hardened and performance using organism-specific signatures will be validated. This study will be designed accordingly to the following critical path, comprising several steps representative of milestones:

PHASE 1 – FEASIBILITY STUDY (12 months)

1. Step 1
    a. Develop and validate the performance of the whole blood, chemiluminescence-based, enhanced solid phase qNPA using model genes
    b. Develop the integrated microfluidic cartridge of the PBA in which to perform enhanced solid phase qNPA assay for rapid detection, utilizing filtration concentration of white cells from whole blood
2. Step 2
    a. Adapt qNPA to the microfluidic cartridge, optimizing the cartridge and the qNPA reagents and protocol
    b. Develop a field-friendly blood collection process and device (collection into anti-coagulant/fixative solution, room temperature storage until testing)
    c. Validate performance: the objectives of the validation relies on assessing the sensitivity to measure white cell genes modulated by infectious organisms from 100 ul blood sample with a turn around time < 6 hours.
    d. Design and prototype the electronic components of the PBA reader

PHASE 2 – DEVELOPMENT (24 months)

3. Step 3
    a. Develop a mobile PBA instrument for controlling the actuators/sensors for automated processing onto the cartridge; miniaturization of the chemiluminescence detection module
    b. Develop a data communications and interpretation software package
    c. Develop the array printing and assembly SOP's for manufacture of the cartridge arrays

    d. Develop an ultra-bright (multiply HRP labeled) detection probe, and evaluate ultra-bright fluorescent probes to optimize sensitivity and turn around time
4. Step 4
    a. Field harden the reagents (convert reagents stored frozen to dry or room temperature and sterile storage)
    b. Field harden the PBA instrument and system
    c. Establish GMP production of the hardware and reagents
    d. Validate the diagnostic gene signatures putatively identified by high density array studies using ArrayPlate™ qNPA™ and fresh and fixed archival tissue
5. Step 5
    a. Demonstrate diagnostic equivalence of the ArrayPlate™ qNPA™ (with mixing method) on which the signatures are validated to the production platform and enhanced solid phase qNPA assay
    b. Obtain UL rating and CE Mark
6. Step 6
    a. Prepare for FDA 21 CFR part 11 compliance
    b. B-test in the field for diagnosis of common infections agents
    c. Establish regional field technical support
7. Step 7
    a. Launch product into the field
    b. Prepare product for acquisition under governmental programs (e.g. Bioshield or the like)

Alternative approaches to potential challenges:
1. Utilize magnetic bead concentration/separation of white cells from whole blood
2. Switch to fluorescent, electroluminescent or Raman probes
3. Instrumental re-design requiring incorporation of excitation sources and potential need for higher power sources (e.g. generating >75W) or customized solid state light emitting sources (e.g. Super Bright LED’s)
4. If fixed blood archived samples (typically ethanol fixed rather than formalin fixed) cannot be tested successfully, then other means of validating the signatures as a function of different infections agents need to be identified, namely collecting frozen or formalin fixed samples from exposed persons accrued over the course of this development program.

Although direct pathogen detection will be enabled by our array-based qNPA approach, host cell detection will also be enabled and will require accessing a set of representative genes. Samples from persons who have been exposed to each infectious agent are required. The identification of putative gene expression biomarkers (by the ongoing EOS high density array program) is required. It is anticipated that archived patient samples can be used for validation of gene signatures. Furthermore, if the ongoing high density array program is not successful, if fresh/frozen samples from patients exposed to bioagents cannot be obtained for the ongoing high density array program, or if archived fixed samples cannot be successfully assayed by the ongoing high density array program,

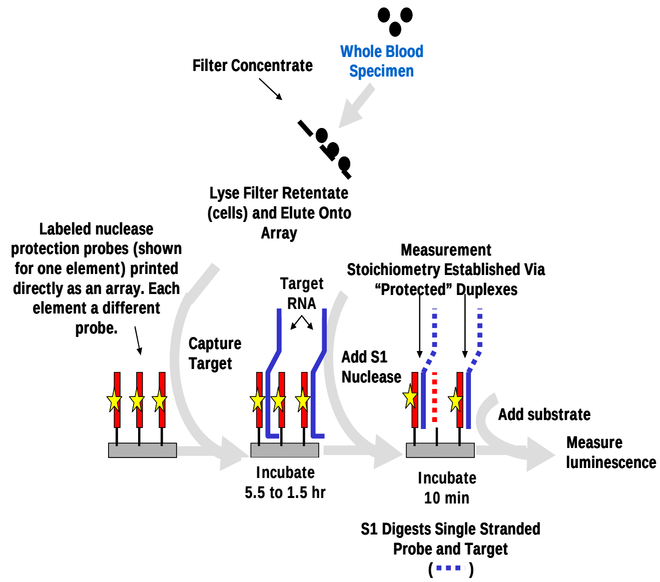
then an alternative is to develop and utilize a whole genome high density array qNPA assay, which can utilize fixed archived samples and provide the identical data, with identical high sensitivity (conserving sample) as from fresh or frozen clinical samples.

The final fielding phase will require addressing the following potential issues:

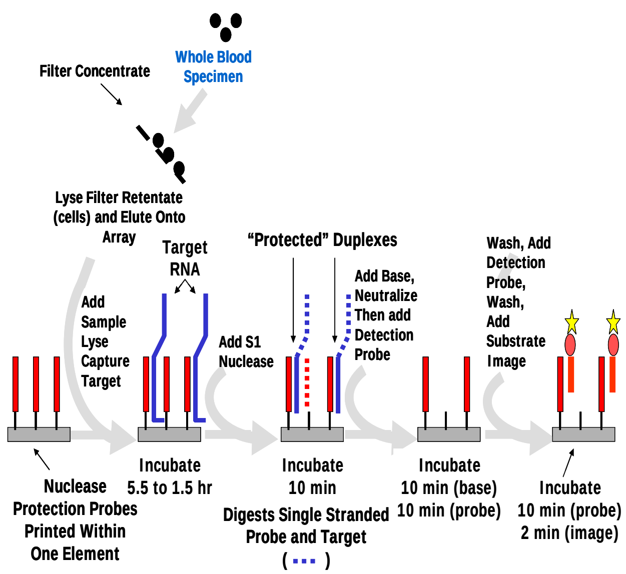
1. The test is likely to require FDA qualification, but not necessarily FDA approval as a diagnostic if it is initially launched as a prognostic assay.
2. There will need to be a technical field support force, ideally distributed regionally to address user issues. Personnel operating the system will require minimal training.
3. There will need to be process development to adapt the storage conditions of the reagents as currently used (e.g. stored frozen) to field compatible methods, (e.g. storage as a solid, dry, or wet at room temperature; preferably on-chip). Production will also have to be GMP.
4. It will be desirable for the units to report results to a central monitoring system, as well as to the user, necessitating a communications package. If the capability of two-way communication is incorporated, centrally located medical and technical experts can review the results. Advice can be given to the user when results are out of the expected range, such as may be encountered upon exposure of persons to a new agent not anticipated or for which there is no signature, or where there was minimal or no availability to archived samples for the development and validation of a diagnostic signature for that organism.
5. Though not required, obtaining UL certification and CE Mark will address safety standards. The collection of blood will be performed in accordance with current DoD collection standards. Note that the sample could also comprise saliva but that may require some further development and re-formulation.
6. The software will need upgrading as new signatures are added or new response information is obtained with actual use.
7. The hardware, reagents, and test protocol will need to be hardened.

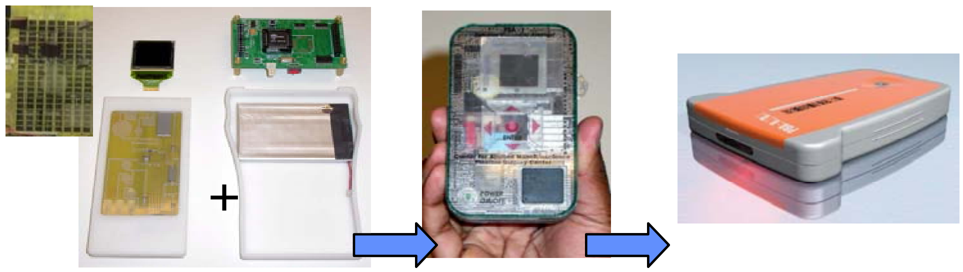
FIGURES

Enhanced Solid Phase qNPA, Primary Scheme



Enhanced Solid Phase qNPA, Alternate Scheme





Future concept

<u>Relevance</u>: The direct qNPA assay has the advantage of non-amplification, high sensitivity, speed, specificity, as well as a low rate of false-positive and false-negative results. This fully automated system with integrated sample preparation has the advantage that untrained users could employ the system, following simple instructions. Renewable plastic-cartridges with on-board reagents will be used to perform the assay, which decreases cost and instrument complexity (no manual re-loading necessary). The proposed system will be able to interface with all current sample collection methodologies (air-borne particle collectors like cyclones, swab-based environmental detection etc.). The use of protein markers for toxins allows for a single platform to test for all three pathogen classes.

<u>ESTIMATE OF COST/SCHEDULE REQUIRED TO FULLY FIELD</u>

The resource requirements will comprise:

- Manpower: $ 8,000,000 for development of a fully functional prototype suitable for manufacture.
- Cost to develop/field: compliant scale-up will require large GMP manufacturing and commercialization similarly to common medical devices development cost practiced by the industry
- Costs and manpower necessary if solution must be integrated into an existing system: tbd / The TBA is a stand-alone system. The only integration is of the EOS program signatures onto the PBA and of the PBA communication system into the shipboard global communication system.

<u>ESTIMATE OF COST TO SUSTAIN ONCE FIELDED</u>

- Manpower requirements
  - Technical support (re-calibration of optics due to shocks, instrument)
- Life-Cycle costs: TBD

- o 1-2 years shelf-life for consumables
- o Instrument > 5-10 yrs

- O&M costs:

- o We anticipate the need to conduct 100,000 tests to validate the prototype. These tests would require high volume (reagents ~ $1/run); Substrate and S1 are expensive components as well as printing costs for fixed array (for 500 elements costs ~$10 using the current printing hardware but should decrease with new manufacturing processing); cartridge will cost ~ $10/unit. R&D cost for 1000 cartridges would be ~ $ 50-100/cartidge
- o Batteries $ 50-100/unit-yr

Solicitation Number: FA7014-07-R-0003 (Epidemic Outbreak Surveillance Advanced Diagnostics Laboratory Support (EOS ADL)

Title: Development of a Universally Deployable Molecular Diagnostic System (OmniDx) by Osmetech Molecular Diagnostics

Contact: Hans Fuernkranz
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Date: **April 17, 2007**

1. Introduction

1.1 The Challenge

It has been nearly 15 years since biological weapons have become a significant national security preoccupation. The Strategic Studies Institute of the US Army War College estimates that almost $30 billion in federal expenditure has been appropriated to counter the anticipated threat. Still, serious gaps remain for the much needed technologies required to reliably detect and analyze actual threats in the field. The currently available field evaluation technology lacks specificity and sensitivity, thus causing confusion in the field for critical decision making. Such technology can result in false positive and false negative results due to the lack of specificity and sensitivity, thus failing in its mission to save lives, protect our armed forces, and address National Security concerns. Consequently, biowarfare and bioterrorism (BW, BT) agents of high regret - those that generate long-term or irreversible impacts on operations or that have potentially severe impacts on personnel – continue to pose a great risk to global force protection and readiness. These agents are typically highly pathogenic organisms with the potential for high morbidity and mortality that can have a significant public health impact, leading to societal and economic turmoil and the lack of efficient functioning of critical infrastructure. The key to controlling the impact resulting from a bioterrorism attack is to detect or identify an agent or outbreak rapidly and reliably so that rapid intervention can be instituted for event mitigation. The ability to identify BW/BT pathogens in near real time is essential to minimize the loss of life. This capability enables rapid clinical intervention and post exposure prophylaxis, initiation of isolation and quarantine of exposed individuals in cases where a contagious or a highly transmissible disease is suspected, avoidance of further spreading of disease to troops and civilian populations, and decision making for evacuation to minimize any additional exposure.

1.2 The Solution

To address this critical need, we plan to develop the OmniDx, a small, fast, easy to use, highly portable robust pathogen detection system with high specificity and sensitivity for use anywhere a BW/BT threat may be of concern – in the battle field, on ships, in field hospitals, at border crossings, in airports and ports, at military bases, and in more traditional settings such as CDC Laboratory Response Network (LRN) labs, state and local public health labs, hospital labs and clinics, and first responder situations. The OmniDx will allow for automated sample preparation and the parallel processing of up to 72 tests per sample. This system will also be uniquely positioned for distributed diagnostic testing, such as the identification of pathogens causing for upper respiratory infections, sepsis, gastric diseases, and drug resistant infections. The OmniDx will be developed under Quality System Regulation (QSR) and will be an FDA approved device.

Because the OmniDx is based mostly on existing technology, we feel that it can be developed within a reasonable time line. This innovation will result from leveraging, combining, and integrating existing technologies into a small, robust system that can be used by non-laboratory personnel with minimum direction and training.

Samples that may contain BW/BT agents or infectious pathogens can be quite varied. They can be powders or liquids that come from environmental sources or samples from human patients, such as blood, serum, and respiratory secretion. One typical collection device that is common for both environmental and patient samples is a swab, where the sample material is collected on the tip of the swab then transferred into a liquid medium for extraction and processing prior to detection. To address the wide variety of sample matrices, the OmniDx will consist of two modules as shown in Figure 1 – one to extract and process the sample and a second for detection. The expected time to answer, beginning with the addition of the unprocessed sample to the sample prep module and ending with the result reported to the user, is under ninety minutes.

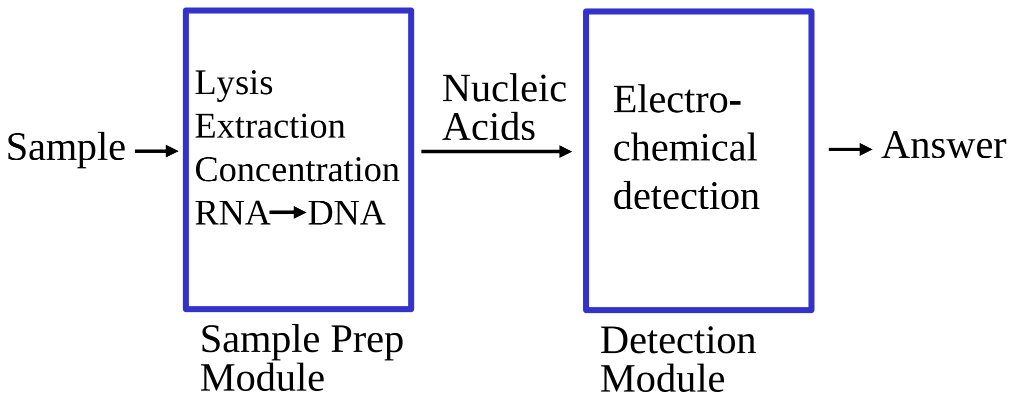

Figure 1: Schematic of the system

The sample prep module will perform lysis of the pathogens, extraction and concentration of the nucleic acids, and perform reverse transcription to create cDNA if needed. The concept for the design of this module may change depending on the end user needs for sample type and volume and the sample collection device. The first system to be developed will focus on white powder incidents, where the sample is collected via a swab to ensure the presence of sufficient target pathogen for analysis and optimization. This will also allow for smaller sample volumes to be used in the first generation product.

The detection module, which will remain unchanged for each sample type, will be based on established electrochemistry technology. Since this is a non-optical detection method, the technology will allow for a portable concept that is robust, has high specificity and sensitivity, and provides cost benefits. An added critical advantage for electrochemical based detection is that it allows for a high degree of multiplexing capabilities, which enables the end user to screen or detect multiple pathogens simultaneously for each sample. This multiplexing capability also allows for the inclusion of positive and negative controls for each sample, which greatly increases the accuracy of and confidence in the results through quality control and assurance. The electrochemical detection technology, which is incorporated into Osmetech's eSensor® Cystic Fibrosis Carrier Detection System, has been approved by the FDA for use in the clinical environment to detect carrier status of cystic fibrosis of adult couples contemplating pregnancy. This FDA-approved product demonstrates that this technology is acceptable to the FDA for clinical use and should ease the approval of future electrochemistry-based products.

1.3 Leverage to Non-Military Applications

The development of the capability to perform sophisticated, highly specific and sensitive tests for the detection of BW/BT pathogens to ascertain critical information pertaining to exposure, disease or health status in an easy to use system will revolutionize our current approach to public health preparedness, force protection and countering terrorism to support national security missions. Future market opportunities include disease detection and identification; response to natural disasters, emerging diseases and other large scale emergencies; food, animal and environmental testing; and ultimately point of care testing. The proposed technology will by definition be very suitable to analyze upper respiratory infections, especially influenza-like infections including bird flu (H5N1) as well as lower respiratory infections if appropriate samples are available. The system will also be very suitable to analyze other clinically significant diseases, such as sepsis and gastric infections, which can be caused by both viruses and bacteria.

Validation of this simple, robust, rugged, easily deployable system through use by military or other personnel will be a very important first step in understanding the benefits, applicability, utility, and limitations of the system for particular applications. This will enable rapid optimization and modification of the system, ultimately speeding adoption for civilian sector use.

Our portable pathogen identification system will be well-suited to become standard equipment for point of care applications in intensive care units, emergency rooms and admittance areas of hospitals. One example of a key civilian market opportunity, used here as an illustration of the utility of such a system, is the detection of methicillin resistant *Staphylococcus aureus* (MRSA), which poses a significant concern for clinical and nosocomial infection in health care settings. The organism *Staphylococcus aureus* is extremely common in the environment and can be carried by many people in the nasal passages, where it causes no major harm to healthy individuals. However, some strains of *Staphylococcus aureus* have exhibited resistance to common beta lactam antibiotics like penicillin. Recently, strains of this bacterium have acquired resistance to more modern antibiotics such as methicillin and vancomycin, and these are referred to as MRSA or VRSA. It is possible to determine whether a strain of *Staphylococcus aureus* is drug resistant or not through the detection and evaluation of targeted genomic or plasmid regions that confer resistance.

According to CDC data, MRSA infections have grown from two percent of the total number of staphylococcus infections in 1974 to 63% in 2004. Patients infected with antibiotic-resistant organisms like MRSA are more likely to have longer and more expensive hospital stays, and may be at high risk for mortality as a result of the infection if not detected and dealt with early. Additionally, when the drug of choice for treating a patient's infection doesn't work, the patient requires treatment with second- or third-choice medicines that may be less effective, more toxic and more expensive in some cases. When patients with MRSA are discovered in a hospital, the hospital will usually try to prevent the infection from spreading to other patients by isolating infected individuals, which is costly and can be

disruptive. Typical MRSA tests take 2-3 days to get a result, by which time additional patients may be at risk for infection and pose a legal liability for the care provider.

The ability to rapidly and easily identify an infection or its spread is key to the effective disease control and prevention of hospital-acquired infections. Identification of the pathogen responsible facilitates the appropriate treatment of the underlying cause and leads to implementation of appropriate measures for containment and prevention. A simple to use panel of diagnostic tests to be employed during the patient admittance process to identify incoming patients with communicable or transmissible disease, during initial patient screening for triage, in support of rapid clinical intervention, or on already admitted patients to determine the source of the infection (fungi, bacteria, viruses) will revolutionized health care practice and provide health care workers with the tools they need to control the spread of infection. The key to such concepts of operation rely upon the test technology being fast and easy to run by non-laboratory personnel, making the proposed system to be developed an excellent and revolutionary tool for this and other distributed testing applications.

2. System Description

2.1 Overview

The OmniDx, will consist of two instrument modules as mentioned above – a sample prep module and a detection module, accompanied by three consumables, a very simple graphical user interface, and data analysis software. It is our intent to keep the instrument modules and the consumables inexpensive, thus increasing the potential for adoption, utilization and maintenance of the system. The OmniDx will be designed such that the consumables fit within the modules only in the correct orientation (thus making it fool-proof), and the software will lead the user through the minimal user interaction required to operate the system. The system will be designed to be easily portable – both instrument modules will fit within a space no larger than a typical briefcase – i.e. about 12" by 18" by 6", and will collectively weigh less than 25 pounds.

2.2 Sample Prep Module

The sample prep module will account for the incorporation or loading of the sample, lysis of any pathogens present in the sample, extraction and concentration of the nucleic acid, and if necessary conversion of the RNA to DNA through reverse transcription. The output of the sample prep module will be DNA in solution that is ready for amplification and detection. The transfer of the DNA from the sample prep module to the detection module will be done manually with the use of a simple transfer device. The design of this transfer device will make it impossible to transfer the solution incorrectly. The intent of this initiative is for this sample prep module to initially be focused on environmental white powder samples.

Pathogen lysis can be done using a variety of currently available methods, either through chemical or mechanical means. Although chemical lysis can be quite effective, it is usually necessary to modify the chemical mixture depending on the types of pathogens expected or anticipated for nucleic acid extraction from a sample. Mechanical methods can be more general, and one that has been shown to be effective is the application of directed ultrasonic

energy. The transducers for this method can be made quite small, and are generally inexpensive.

Similarly, there are many available methods for concentrating or capturing nucleic acid after extraction, including capture of the nucleic acids on membranes or beads. The use of magnetic beads as the capture material is the better choice for this application, as it is possible to capture the nucleic acid from a large sample volume, pull the beads together into a small volume with a magnet, and elute the nucleic acid into a small volume of sample, thus effectively concentrating the extracted nucleic acids. The smaller, more concentrated sample enables a smaller detection unit with faster amplification and enhanced sensitivity. Once the nucleic acid is eluted from the beads, a reverse transcription step will be performed to convert any RNA in the sample to cDNA. Any and all reagents necessary for the sample prep step will be contained within an inexpensive consumable in a stable form and will not need to be added or mixed by the end user, thus reducing the work load and the possibility of human error.

2.3 Detection Module

The optimal method for pathogen detection and identification is through the detection of specific sequences of nucleic acid (DNA or RNA, depending on the pathogen).   One typically used detection method is amplification of the nucleic acid using polymerase chain reaction (PCR) for DNA or Reverse Transcription PCR (RT-PCR) for RNA coupled with detection of specific target nucleic acid sequences through the use of probes that bind to the amplified target sequence region. Often the bound probes are detected with fluorescence, requiring relatively expensive and not very robust optical systems that must be calibrated on a periodic basis to optimize the performance of the instrument to yield quality results. Additionally, the number of pathogens in a given sample that can be detected simultaneously is limited to four or five because of spectral overlap of the emission wavelengths of the fluorescent dyes. One method used to overcome this limitation is to split the sample into individual wells or tubes and perform amplification and detection analysis separately. However, this reduces the sensitivity of the detection because the amount of pathogen present in each tube or well is reduced by a factor equal to the number of times the sample is split. It also slows the testing (as time must be taken to measure each reaction separately) or increases the cost of the instrument because of the need for parallel optical channels, requires more reagents, and lacks the ability to detect for multiple pathogens at a given time.

It is possible to achieve a much higher degree of multiplexing by designing single stranded oligo probes that are present in solution during the PCR and have the ability to hybridize to capture oligos on an electrode as target amplification occurs. The location of the bound probe on the surface is indicative of the presence of a target specific sequence of nucleic acid associated with a particular pathogen of interest in the sample. The cleaved probe bound to the surface can then be detected using a variety of methods. A very simple, robust detection method is based on electrochemistry, where the capture nucleic acid is attached to an electrode and any cleaved probe that binds to the complementary nucleic acid on the surface is detected by measuring a current. This method enables the detection of multiple pathogens simultaneously in a rugged, compact, inexpensive package. The system to be developed under this proposal is based on this electrochemical detection method.

In order to take full advantage of the multiplexing potential that electrochemical detection affords, it is necessary to hybridize potential target nucleic acids to an array of electrodes modified with unique capture probes. This necessitates that there is a single stranded target available after the amplification step. One way to achieve this is to utilize asymmetric PCR, as shown in Figure 2. In this amplification strategy, the forward and reverse primers are not at equimolar concentrations in the PCR reaction. As the exponential amplification proceeds, one primer is exhausted first and the other primer then starts producing single stranded amplicons in a linear fashion. This single stranded species can then be hybridized to a capture probe at the electrode at a region interior to the primer sites. This method retains excellent specificity due to the three distinctive hybridization steps and allows for detection without any post-PCR treatment of the solution making this method suitable for a totally enclosed chip based assay.

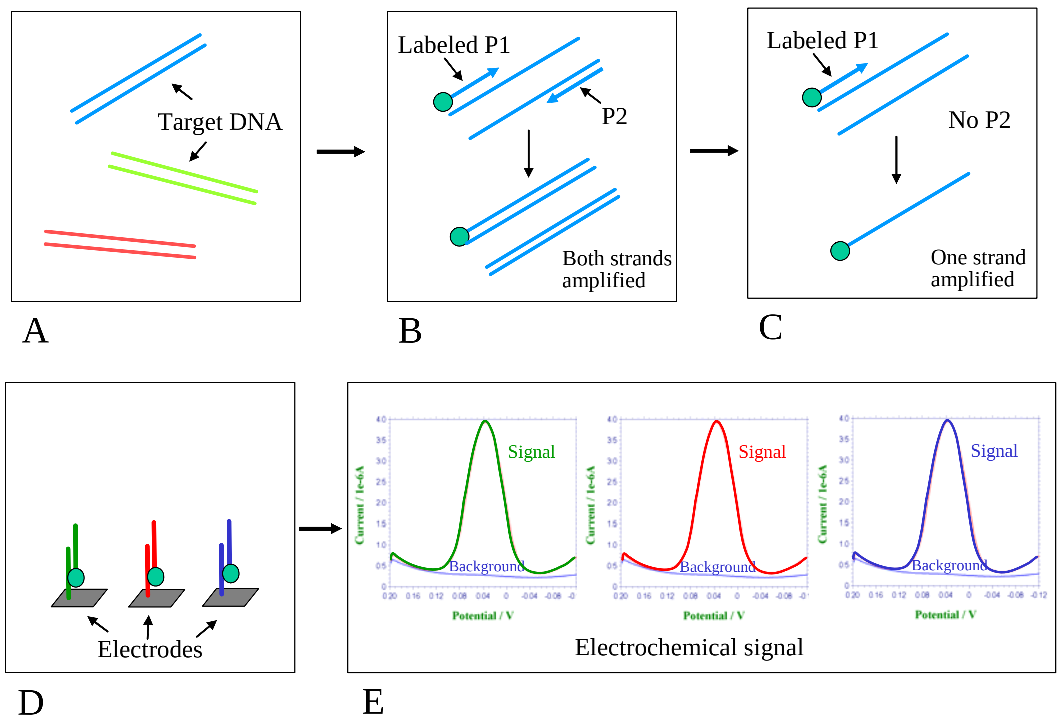

Figure 2: Schematic of the asymmetric PCR, electrochemical detection. (A) DNA in the sample, where the color is representative of different targets. (B) The first stage of asymmetric PCR amplification, where the two primers (P1, with an electrochemical label, and P2) are present in solution. Only amplification of one target is shown for simplicity. (C) The second stage of asymmetric PCR, where only P1 is present, and a single stranded amplicon is generated. (D) The single stranded, labeled amplicon binds to a capture oligo attached to an electrode. (E) When a voltage is applied, a current is measured for each electrode.

The modular design of the OmniDx will make decontamination easy and fast. The "closed system" approach to this design ensures not only safe operation but also eliminates the risk of amplicon contamination. The user will transfer the DNA sample from the sample prep module to the detection module via a fully enclosed cartridge. The DNA will then be

amplified prior to detection of the nucleic acid. This amplification can either be accomplished by PCR, which requires up to 40 cycles of thermal cycling, or through isothermal amplification. Thermal cycling can be completed quite quickly on small volumes of fluid because of the low thermal mass. There are many existing methods by which to cycle the temperature, including moving the fluid back and forth between different temperature zones or keeping the fluid stationary and heating and cooling with a device such as a peltier.

It is also possible to amplify without thermal cycling by using a number of existing isothermal methods. With this type of amplification, it is possible to simplify the design of the instrument because the power requirements are typically less than those needed for regular thermal cycling concepts. However, it is possible that more reagents may be necessary in comparison to PCR. The system to be developed under this proposal could incorporate either amplification method based on end user input supported by trade-off studies.

The DNA sample will be contained within the reader module in a disposable plastic consumable that will contain all of the necessary reagents in dry form. This consumable will also contain channels to control the flow of sample and the electrodes required for the multiplexed electrochemical detection.

The feasibility of electrochemical detection method in a small, self-contained instrument was shown by Robin Hui Liu et. al. in a paper published in 2004[1]. Additionally, Osmetech has shown the feasibility of electrochemical detection for genotyping of the CYP450 2C9 *2 (430 C>T) polymorphism using eSensor® mixing cartridges. As shown in Figure 3, genomic DNA or cloned amplicon samples were amplified in a multiplex PCR process generating seven amplicons and using either a 100-fold excess of target-strand primers (asymmetric PCR, panel A) or equal amounts of target and non-target strand primers (symmetric PCR, panel B). In the case of symmetric PCR in Panel B, the amplification product was subjected to digestion with bacteriophage λ exonuclease (20 min at 37ºC) to destroy the 5'-phosphate tagged non-target amplicon strand. The products of PCR amplification (and exonuclease digestion, where applicable) were then directly mixed with hybridization buffers containing ferrocene-labeled major- and minor-allele signal probes for 10 polymorphisms of the CYP450 2C9 and the promoter polymorphism of the VKORC1 gene and applied directly to eSensor® mixing cartridges. The cartridges were processed for 30 min at 40ºC to allow hybridization, and then the electrodes were scanned to determine bound label. Samples were a mixture of excess patient genomic DNA specimens derived from whole blood, cell line genomic DNA samples (from the Coriell Cell Repositories) and cloned wild-type or mutant amplicons.

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[1] R.H. Liu *et. al.*, "Self-Contained, Fully Integrated Biochip for Sample Preparation, Polymerase Chain Reaction Amplification, and DNA Microarray Detection," *Anal. Chem.*, **2004**, *76*, 1824-1831.

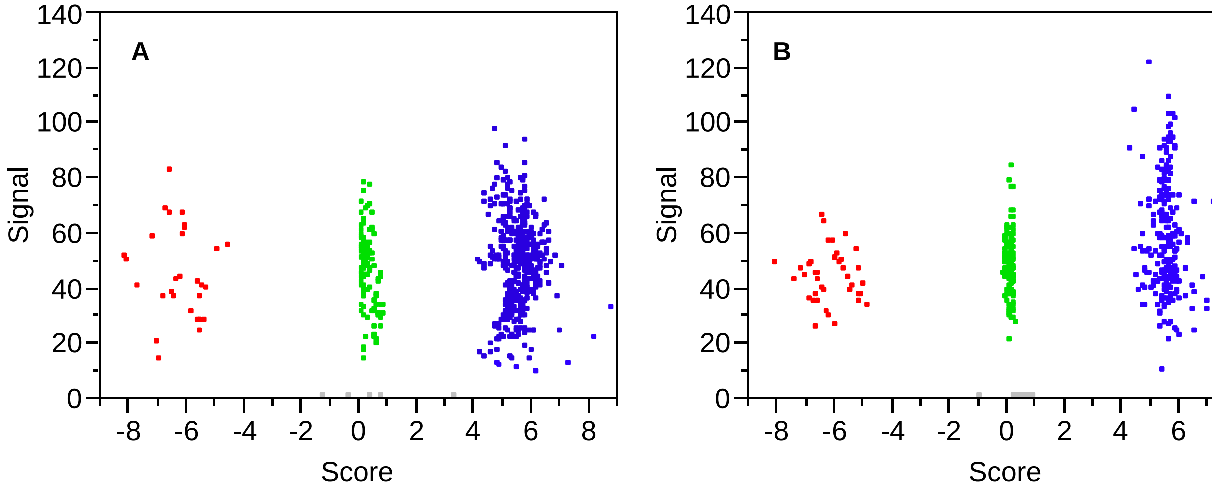

Figure 3: Sample genotypes were either homozygous major allele (blue symbols), heterozygote (green symbols), or homozygous minor allele (red symbols) genotype and were confirmed by DNA sequencing; all heterozygote samples were from genomic DNA. Data were from 175 (asymmetric PCR, panel A) or 108 (symmetric PCR/exonuclease digestion, panel B) cartridges; results were obtained for four electrodes per cartridge. Results are plotted as Signal (major allele current + minor allele current) in nA on the Y-axis versus Score [log2(major allele signal/minor allele signal)] on the X-axis. Results were excluded (grey symbols) for signals below 2.5 nA, or for cartridges for which the positive or negative controls failed their specifications (no symbols).

As mentioned above, Osmetech has also commercialized an FDA-approved cystic fibrosis test based on an electrochemical consumable and instrument. This system screens for 23 cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations. Details of the performance of this product can be found at http://www.osmetech.com/pdf/CMS_Poster.pdf. The system to be developed under this proposal will leverage the QSR infrastructure Osmetech has put in place for the development of the FDA- approved cystic fibrosis product.

2.4 Consumables

The OmniDx will include three easy to use, inexpensive consumables. One consumable will be designed for white powder collection, transfer of the solid powder into a solution, transport (if needed), and insertion of the powder suspended in solution into the sample prep module. The second consumable will be for use in the sample prep module and will contain all of the necessary reagents for sample lysis and nucleic acid extraction, concentration, and purification. Once the sample is ready to be transferred to the detection module, this consumable will be removed by the user and used to insert the nucleic acid sample into the detection module. The third consumable will be used in the detection module for amplification and electrochemical detection, and will contain all of the reagents required for these steps. The use of three separate consumables instead of one integrated consumable will allow for the development and manufacture of less complex devices, leading to more robust consumables, higher manufacturing yields, and lower costs.

2.4 Instrument Software and Graphical User Interface

The software will run on a real-time embedded operating system and the interface will be designed with early involvement from the end-user. This user interface will take human and environmental factors into account to make it intuitive and simple to use in field applications.

Where possible, the system design will make use of self-identification in the consumable and self-configuration in the instrument modules to minimize human error. Self-test instrument software will ensure confidence in the instrument hardware. An interface will be available for remote connectivity and operation. The software design will be modular to facilitate development, testing and maintenance. This will also make the software easily extendable to other applications.

2.5 Instrument Hardware
The two instrument modules will be provided in rugged portable enclosures and will be designed to be battery operated, if needed, for field applications. Power management techniques will be used to extend battery life. A hardware connection will be provided to retrieve time-stamped log files that contain relevant information such as results, data provided by the user, reagent and consumable lot numbers and expiration dates, and system status indicators.

2.6 Intellectual Property
In 2005, Osmetech acquired from Motorola a significant IP portfolio covering compositions, methods, materials and devices for electrochemical DNA detection. This IP estate was originally part of CMS, a company founded out of CalTech, which was acquired by Motorola in 2000 for over $200M. During its period of ownership of CMS, Motorola made significant investments in electrochemistry technology, further strengthening and expanding this IP. The rights for the use of TaqMan IP for diagnostic applications are generally available for license from Roche Diagnostics, and Osmetech plans to acquire a non-exclusive license to TaqMan for use with electrochemical detection.

3. Requested Funding and Timeline

Osmetech requests $20M of funding over a 3 year time frame to develop a small, very portable electrochemical sensor device and accompanying sample prep device to the prototype level. The sample preparation module will be designed to handle white powder samples collected on swabs. However, the technology used for this sample prep module can be leveraged for other sample types such as nasal swabs, blood, urine, etc. The detection module will incorporate on-board nucleic acid amplification and an electrochemical-based consumable designed to run 72 tests simultaneously. Three consumables will be developed – one for sample collection and addition to the sample prep module, a second for sample prep that contains all reagents necessary for this step, and a third for the amplification and electrochemical detection that contains all of the reagents required for this step in a dry, stable form. Although the system to be developed will ultimately be of use for many pathogen detection applications, the first assay of interest, which will be prototyped under this proposal, will be a panel of CDC Category A bioterrorism pathogens including anthrax, botulism, plague, and small pox.

This three-year period will consist of two phases. At the end of the first phase, which will have a duration of 15 months after initiation of funding, Osmetech will demonstrate early feasibility for the two instrument modules, three consumables and early-stage software. These components will consist of the required function, but may not have the final form of

the product. The goal will be to demonstrate that the technology can be used to detect a number of pathogens simultaneously in an automated fashion. This phase will require $8M of funding to support approximately 20 full time employees.

During the second phase, which will extend 21 months after completion of the first phase, Osmetech will complete the system to the prototype level. At this point, the system will have both the form and function of the final product, although the components will be built within an R&D environment, not a manufacturing environment. The system will be developed under QRS in preparation for filing for FDA approval. After this 21-month phase, the system will be ready for analytical validation. The analytical validation studies, clinical trials and preparation of the documentation for FDA filing are outside of the scope of this proposal. The second phase will require an additional $12M of funding for personnel, tooling and materials.

4. Personnel

Hans Fuernkranz will lead the development of the OmniDx system. He has over 15 years R&D experience in the pharmaceutical and biotechnology industries. Hans has specialized in the development of new technologies, building and leading interdisciplinary teams of engineers and scientists. Hans' international education and training uniquely blend engineering and science. His technology and product development expertise includes MEMS devices, micro fluidics, hybridization arrays, and bio-assay integration.

Hans' career highlights include:

- R&D experience in companies ranging from biotech start-ups to large pharmaceutical corporations with 20 to 20,000 employees, and budget management of up to $20M.
- Commercialization of a highly successful gene expression microfluidic card product for Applied Biosystems in collaboration with 3M.
- Creation of technology and quality systems solutions for the Applied Biosystems AB1700 gene expression hybridization array product.
- Design and implementation of quality systems solutions for Applied Biosystems' Consumables Development & Manufacturing Division, a $650M business at the time.
- Development of proposals and negotiation of license and supply agreements resulting in $25 million, multi-year collaborations.
- Initiation and management of highly successful research collaborations with academic and clinical institutions

Hans is experienced in establishing research capabilities designed to develop technologies in close collaboration with customers. He is a proven leader with a track record in developing and driving compelling new technology/product visions with a history of recruiting and retaining world-class R&D talent.